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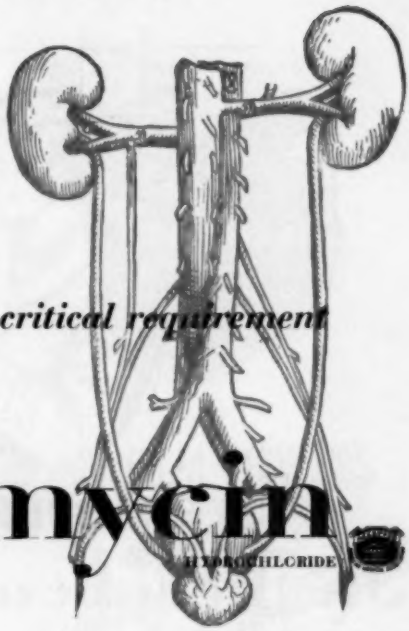

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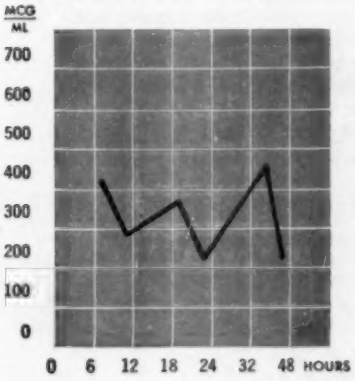

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2. Welch, H., Hendricks, F. D., Price, C. W., and Randall, W. A.: J. A. Ph. A. (Sc. Ed.) 39:185 (Apr.) 1950.
3. Welch, H.: Ann. New York Acad. Sc. 53:253 (Sept. 15) 1950.

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TUBERCULOUS CAVITIES OF THE LOWER LOBE

Results of Treatment in 103 Patients

JOHN S. CHAMBERS, JR.

(Received for publication February 26, 1951)

INTRODUCTION

Cavitary tuberculosis of the lower lobes has presented a problem in management since the inception of collapse therapy in pulmonary tuberculosis. There has been great variation in opinion as to what methods of treatment give the best results. With the newer means of treatment available in the last five to ten years, such as resection, pneumoperitoneum, and streptomycin, it was believed that a study of a group of patients with lower lobe cavitary tuberculosis largely treated by the older methods was essential to evaluation of the new methods.

Composition of Clinical Group

The material in the study was selected by reviewing the chest roentgenograms of 3,174 patients admitted to the Michigan State Sanatorium from January 1, 1937 to January 1, 1947. Another group of patients was selected by reviewing chest roentgenograms and records at the University of Michigan Hospital, with the result that a combined group of 103 cases has been collected for study, 76 of which were from the Michigan State Sanatorium and 27 from the University Hospital. All patients were chosen for study who presented: (a) a lower lobe cavity with tuberculosis confined to that lobe; and (b) lesions in other lobes, but with the lower lobe cavity constituting the principal problem in therapy. Patients who did not fall into these two categories were listed for statistical analysis of the incidence of lower lobe cavities in a sanatorium population.

History

From the time of Laennec until the nineteen twenties it was generally taught that pulmonary tuberculosis had its inception in, and mainly affected, the apex of the lung, and progressed downward only in the terminal stages. Kidd (1) in 1886, however, realized that the apex of the lower lobe "may be occasionally attacked before the apex of the upper lobe." Fowler (2) in 1888 stated that "the upper and posterior part of the lower lobe is a spot only second in vulnerability to the apex itself." The writings of Kidd and

[1] From the Department of Surgery, University of Michigan, Ann Arbor, and the Michigan State Sanatorium, Howell, Michigan.

Fowler apparently went unheeded for twenty or twenty-five years. Norris and Landis (3) in 1920 wrote concerning pulmonary tuberculosis that "it almost invariably starts at the apex and extends downward." Fishberg (4) in 1922, after stating that basal lesions were "extremely rare," indicated that when they did occur they were "terminal phenomena." These opinions gained widespread acceptance despite the paper of Gekler (5) in 1917 regarding acute subapical disease and the work of Assmann (6), Redeker (7), and Wessler and Jaches (8) amplifying the subapical concept.

Pohl (9) and Fishberg (4) in 1928 stressed the difficulty in discovering lesions in the lower lobes because of obscuration of this part of the lung by the hilum and heart in the usual postero-anterior roentgenograms. This point is not fully appreciated today, as is illustrated by the reluctance of many phthisiologists to use lateral and oblique roentgenograms. All of the 103 patients analyzed in this presentation had lateral and stereoscopic postero-anterior films at some time during hospitalization.

The work of Sweany, Cook, and Kegerreis (11) in 1931 on the localization of primary cavities in pulmonary tuberculosis graphically illustrated the tendency of the apices of the lower lobes to be involved in cavernous disease. These authors used three-dimensional localization of cavities by means of postero-anterior stereoscopic roentgenograms, and found that 13.7 per cent of cavities in 204 patients were in association with the bronchus of the apex of the lower lobe.

In reviewing the literature on the subject of lower lobe cavitary tuberculosis, one is struck with the paucity of articles referring to this subject. There are many papers, however, dealing with closely related conditions. Some writers selected cases for study by excluding all lesions above the hilum, thus excluding some cases in which the apex of the lower lobe was involved. Others included middle and upper lobe lesions provided that they were below the hilum or mid-chest. Some authors studied disease localized to only one portion of the lung and others included cases in which several portions of the lung were involved in a more widespread manner, the base of the lung representing the major problem. In all series found in reviewing the literature, minimal or exudative noncavitary disease has been included with cavitary disease. The sole exception was Crofton's excellent article on cavities in the apex of the lower lobe (37). Weidman and Campbell (12), and Busby (13), for example, selected *cavernous and noncavernous* disease in the lower lobe. Romendick, Friedman, and Schwartz (14) studied cavitary and noncavitary disease in the *lower half of the lung field*. Hawkins and Thomas (15) do not define "basal tuberculosis" at all. Although this makes for confusion in comparing various series, the variation is probably more apparent than real when two facts are kept in mind: Most lower lung field tuberculosis is in the posterior chest and therefore in the lower lobe (11); and lower lung field disease tends to excavate early (28).

Pathogenesis

There is no satisfactory explanation for the types of localization of tuberculosis in the lungs. Dock (16) suggests that the apical and posterior portions of the lung receive less blood supply and are thus more prone to infection. Lower lobe tuberculosis has been observed to be associated with pleurisy with effusion by Erwin (36), and by Hawkins and Thomas (15). Erosion of tuberculous nodes at the hilum has also been associated with primary lower lobe tuberculosis (17, 18). Another theory explaining the more frequent onset of tuberculosis in the lower lobes in women than in men has to do with the fact that they breathe more costally than do men. Viswanathan (20) believed that the tight loin cloth

worn in India was a factor in Indians' high incidence of lower lobe disease. The high diaphragm and costal breathing of pregnancy are used by Reisner (10) as an explanation of the high incidence of lower lobe disease in pregnant women. As yet, no satisfactory evidence relating the onset of tuberculosis to location in the lung has been brought forth.

OBSERVATIONS

Incidence

During the ten-year period from January 1, 1937 to January 1, 1947, 2,162 patients with moderately advanced or far advanced reinfection type of pulmonary tuberculosis were admitted to the Michigan State Sanatorium. Of this group, 386 (17.9 per cent) had roentgenographic evidence of lower lobe cavity. Since the criteria for the classifications "moderately advanced" and "far advanced" do not necessarily require the presence of a cavity, fewer than 2,162 patients had cavitary tuberculosis. It can be estimated, therefore, that the incidence of lower lobe cavity in cavitary pulmonary tuberculosis is probably between 20 and 25 per cent in this tuberculosis hospital.

The above figures represent, if anything, a conservative estimate of the incidence of lower lobe cavity in a tuberculosis hospital population. Many lower lobe cavities were presumably overlooked in this survey since lateral roentgenograms were not made routinely on admission, and even stereoscopic postero-anterior roentgenograms were not obtained routinely for a short period during World War II. Certain lower lobe cavities, especially those behind the heart, cannot be demonstrated roentgenographically without lateral or oblique or postero-anterior Potter-Bucky films, and remained undetected because of their location behind the heart and lung roots. It is well known that the so-called "hilar" cavity almost always represents a cavity in the posterior lung field and is, therefore, located in the apex of the lower lobe.

Among the 386 patients at the Michigan State Sanatorium in whom a lower lobe cavity was discovered, the great majority presented bilateral far advanced disease or were obviously terminal cases on admission. Thirty-two patients, however, had tuberculosis localized to one lower lobe at the inception of treatment. Another 44 patients had so little disease in other lobes that they could be included in the group for study since the lower lobe cavity presented the principal therapeutic problem. Thus, 76 cases were collected from the 3,174 admissions for study. This is an incidence of 2.4 per cent of sanatorium admissions which is essentially the incidence of more or less localized basal tuberculosis mentioned in the literature. Thus, Andosca and Foley (21) in 1942 found that 32 of 3,103 patients had basal tuberculosis after the exclusion of patients with more widespread disease. Others mention 2.7 per cent (14); 1.7 per cent (22); 1:1,300 patients; and 6.4 per cent (23).

Ross (24), however, found that 19 of 60 nurses had "hilar" or basal lesions, suggesting that lower lobe lesions may be more common in women. Among the total group of 57 patients with lower lobe cavity from the Michigan State Sanatorium and the University Hospital, with lesions confined to one lower lobe, 24

were males and 33 females. This confirms previous findings (10) that females are more prone to lower lobar disease.

Race cannot be evaluated in this series. Of the 57 patients, with but two exceptions, all were white. This parallels previous series, although Dunham and Norton (25) had a high percentage of Negroes in their series from a tuberculosis hospital with a large Negro population. Viswanathan (20) found among inhabitants of India 6.4 per cent of cases in which the lower lobe was involved. Tirona (17) reported an incidence of 1:1,300 in the Philippines.

The age distribution by decades in the present series is shown in table 1.

Pregnancy is mentioned by some authors as predisposing to lower lobe disease. Four of 28 of Reisner's (10) women patients with lower lobe disease were pregnant. In the present group of 57 patients with solitary lower lobar cavity, 7 of the 33 women patients had the disease associated with pregnancy.

TABLE 1

Age Distribution of 103 Patients with Tuberculous Cavities in Lower Lobes

AGE	NUMBER OF PATIENTS		TOTAL
	Group A (Unilobar Disease)	Group B (Multilobar Disease)	
10–19	10	8	18
20–29	30	21	51
30–39	13	10	23
40–49	1	2	3
50–59	2	2	4
60 and over	1	3	4
	57	46	103

Diabetes is believed by Reisner (10) to be a factor predisposing to lower lobar disease, as 4 of his 55 patients had this complication. There were 2 diabetics in the present series of 57 solitary lower lobar cavity patients.

Diagnosis

It is not difficult to fail to make the diagnosis of tuberculosis in a patient who presents the roentgenographic picture of a cavity in the lower lung field. In 2 of the 57 patients with solitary lower lobe cavity, the diagnosis of tuberculosis was not made for more than a year, despite serial roentgenograms. One patient had open surgical drainage of a supposedly pyogenic abscess and the diagnosis of tuberculosis was not made until a spread of the disease to the contralateral base occurred a year later. The following quotation from Kidd (1) in 1886 cannot be disputed more than a half century later:

"Without attempting to discuss the diagnosis . . . I would strongly urge the importance of routine examination of sputum in all such (basic [sic] disease) cases."

All of the 103 patients in the present study had bacteriologic confirmation of the diagnosis of tuberculosis.

Treatment

The 76 patients from the Michigan State Sanatorium and the 27 patients from the University Hospital in the series analyzed have been divided into two groups: A, cavitary tuberculosis localized to one lower lobe; and B, more widespread tuberculosis with the lower lobe cavity presenting the principal therapeutic problem.

There were 57 patients in the A group and 46 in the B group. Before 1945, phrenic nerve paralysis was less frequently used on these two services than is

TABLE 2

Types of Treatment Used in 103 Patients with Tuberculous Cavities in Lower Lobes

	GROUP A (UNILOBAR DISEASE) SOLITARY TUBERCULOSIS		GROUP B (MULTILOBAR DISEASE) MORE WIDESPREAD TUBERCULOSIS	
Pneumothorax alone		14		15
Phrenic paralysis alone		10		5
Pneumothorax and phrenic paralysis		13		7
Phrenic paralysis and pneumoperitoneum		7		2
Bed rest alone		1		3
Streptomycin alone (only since 1946)		2		0
Resection alone		1		0
Multiple procedures:*		9		14
Including: a. Cavernostomy	3		5	
b. Resection	7		4	
c. Thoracoplasty	3		7	
		57		46

* Several patients had a combination of two or three of the forms of treatment named under multiple procedures and several other patients had a combination of the procedures listed above multiple procedures.

the present practice in the treatment of lower lobe cavities. The majority of patients in this series were treated with pneumothorax. In table 2 may be seen the various forms of treatment which were used in the 103 patients.

The results of the various forms of therapy shown in table 2 have been evaluated as to cavity closure and final status of the patient. The former is an index of the efficacy of immediate treatment, and the latter an index of the long-term result regardless of any additional therapy which may have been necessary. The follow-up period in this series averaged four and one-half years after discharge from the Sanatorium. The average follow-up period is broken down into various groups in table 5.

Since many patients had multiple procedures, separation into groups according to the therapeutic procedure was difficult. This accounts for the apparent discrepancies between table 2 and some of the later tables relating to cavity

closure and final results. For example, a patient who had several temporary phrenic nerve interruptions but failed to attain cavity closure until pneumothorax was instituted is included in the phrenic nerve interruption group for evaluation of cavity closure and in the phrenic paralysis-penumothorax group for final results. In this way a more valid evaluation of each procedure has been obtained.

Pneumothorax

Fifteen patients (Group A) with disease confined to the lower lobe initially had pneumothorax alone. (One patient had bilateral lower lobe cavities and was treated with bilateral pneumothorax.) Sixteen patients (Group B) with more widespread disease had pneumothorax alone.

Of the 16 lower lobe cavities in Group A, only 3 failed to close with pneumothorax. One of the 3 patients had no further treatment and died; 2 had resection of the lower lobe cavity area. In one of these 2 patients, the sputum has become free of tubercle bacilli and the lesion is now inactive; the other patient still has active disease and is in the hospital. In one other patient of the 15 in Group A, the disease is arrested[1], but tubercle bacilli can be cultured occasionally from the sputum, and the patient is at home on limited activity. In the remaining 12 patients, the disease can be classified as inactive 23 to 124 months after discharge from the Sanatorium (average follow-up period, 67.5 months since discharge).

Of the 16 patients in Group B, pneumothorax failed to close the lower lobe cavity in 5. In one, the cavity closed after a thoracoplasty, and 3 could not be treated further and are now dead. Of the 16 patients in Group B, 7 have inactive disease 14 to 137 months after discharge from the Sanatorium (average follow-up period, 85 months after discharge); one died of carcinoma with inactive tuberculosis; 6 died of tuberculosis; and 2 still have active disease (tables 3 and 5).

Complications of pneumothorax: In analyzing the complications of pneumothorax, patients who had both phrenic paralysis and pneumothorax were added to the group with pneumothorax alone. In Group A (unilobar disease) there were 31 patients who were treated by pneumothorax for lower lobe cavity. (One patient had bilateral basal cavities.)

The complications are listed below:

Spread or exacerbation of disease	11 of 31 patients.
(a) Spread of disease on discontinuance of pneumothorax	4 of 26 patients who had the pneumothorax abandoned.
Clear effusion	7 of 32 pneumothoraces.
Empyema	4 of 32 pneumothoraces.
Nonexpandable lung	3 of 26 patients who had the pneumothorax discontinued.

[1] The 1950 National Tuberculosis Association classification of status is used throughout this report. "Inactive" refers to six months of roentgenographic stability and sputum or gastric culture negativity. "Arrested" refers to three months of roentgenographic stability and sputum concentrate negativity.

Deaths due to complication of pneumothorax	2 of 31 patients; one from empyema; one from bilateral spread on expansion of lung.

There were 27 patients in Group B who had pneumothorax, either alone or in conjunction with phrenic nerve interruption. The complications in Group B are listed below:

Spread or exacerbation of disease	9 of 27 patients.
(a) Spread of discontinuance of pneumothorax	3 of 17 patients in whom pneumothorax was discontinued.
Clear effusion	5 of 27 patients.
Empyema	7 of 27 patients.
Nonexpandable lung	3 of 17 patients in whom lung was expanded.
Thickened pleura with impaired function	2 of 17 patients.
Deaths due to complication of pneumothorax	8 of 27 patients; of these 3 were from empyema and 5 from spread

Phrenic Nerve Interruption

In Group A (unilobar disease), 20 patients had phrenic nerve interruption. Forty-seven operations for temporary phrenic nerve interruption were performed on the 20 patients, an average of 2.35 operations per patient. Pneumoperitoneum was used in 6 patients, a phrenic belt in 5 patients, and both pneumoperitoneum and a belt in 2 patients. Cavity closure was not accomplished in 2 patients, one of whom has had no other treatment as yet and is still hospitalized. The other has inactive disease following a resection of the superior segment of the lower lobe. In 2 patients the cavity reopened; one of these patients had a supplementary pneumothorax with closure of the cavity but is still hospitalized, enough time not having elapsed for her disease to be classified as inactive. The other patient with a reopened cavity had a spread of disease and was treated successively with pneumothorax and thoracoplasty, and the disease is now classified as inactive.

Of the 18 patients who had phrenic nerve interruption and no pneumothorax, 14 have inactive disease one to 106 months since discharge from the Sanatorium (average follow-up period, 39.5 months after discharge); 2 have active disease and are in the hospital; and 2, although still hospitalized, have sputum negative for tubercle bacilli but have not completed treatment.

In Group B (multilobar disease), 10 patients had phrenic nerve interruption. The procedure was carried out eighteen times, 1.8 operations per patient. Complementary pneumoperitoneum was used in 3 of the 10 patients. Two of the 3 patients who failed to achieve cavity closure after phrenic paralysis had thoracoplasty and now have inactive disease. One patient had a thoracoplasty and cavernostomy but still has an open cavity with sputum positive for tubercle bacilli. This patient refuses resection and is at home on limited activity.

Of the 10 patients in Group B with phrenic nerve interruption, 7 have inactive disease 10 to 58 months after discharge from the Sanatorium (average of 28.2 months' follow-up after discharge); one is still under treatment, although the

TABLE 3

Cavity Closure in Lower Lobe Tuberculosis*

By Pneumothorax; by Phrenic Paralysis; and by Pneumothorax and Phrenic Paralysis

	PATIENTS	CLOSED	UNCLOSED	FURTHER TREATMENT	NO FURTHER TREATMENT
Pneumothorax					
Group A	16†	13	3	2 (resection)	1
Group B	16	11	5	2 (1 phrenic 1 thoracoplasty)	3
Total	32	24	8	4	4
Phrenic paralysis					
Group A	20	18	2	1 (resection)	1
Group B	10	7‡	3	3 (2 thoracoplasty; 1 thoracoplasty and cavernostomy)	0
Total	30	25	5	4	1
Pneumothorax and phrenic paralysis					
Group A	16	14§	2	2 (thoracoplasty)	.0
Group B	11	7‖	4	3 (2 thoracoplasty; 1 resection)	1
Total	27	21	6	5	1

* Cavity closure was determined by roentgenographic means alone, by means of special films such as laminagrams, Potter-Bucky films, etc., but more often by postero-anterior stereoscopic films.

† There were actually 15 patients, but one patient had bilateral pneumothorax for bilateral lower lobe cavities.

‡ One patient, though the cavity closed, required resection of the blocked cavity in the atelectatic, bronchiectatic lower lobe before reaching inactive status.

§ One patient, though the cavity closed, required resection of a bronchiectatic lower lobe before reaching inactive status.

‖ One patient, though the cavity became blocked, required resection for conversion of sputum.

sputum is negative for tubercle bacilli; and 2 have active lesions. One of these patients had inactive disease for three years but is now rehospitalized with a spread of the disease. The other is at home and refuses resection (tables 3 and 5).

Of the 28 patients with phrenic nerve interruption in Groups A and B, there

was spread or reactivation of disease in 3 patients and permanent paralysis of the hemidiaphragm in 2 patients. In the pneumothorax-phrenic series, one other patient had a permanent paralysis. Thus, 3 (5.5 per cent) of 55 patients who had phrenic operations had permanent paralysis although temporary phrenic nerve paralysis was intended. It is probable that the percentage is actually higher since in the majority of the patients there was no notation on the chart concerning the function of the diaphragm by fluoroscopy at the time of discharge from the hospital or on follow-up examinations.

TABLE 4
*Cavity Size**

	NUMBER OF PATIENTS		
	Less than 3 cm.	3 to 5 cm.	More than 5 cm.
Pneumothorax			
Group A (unilobar disease)	10	3	3
Group B (multilobar disease)	7	5	4
Total	17	8	7
Phrenic paralysis			
Group A	13	3	2
Group B	6	0	4
Total	19	3	6
Pneumothorax and phrenic paralysis			
Group A	10	3	3
Group B	5	1	5
Total	15	4	8

* Cavity size was measured on postero-anterior roentgenograms by means of a centimeter rule.

Pneumothorax and Phrenic Nerve Interruption

Sixteen patients in Group A (unilobar disease) and 11 patients in Group B (multilobar disease) were treated with pneumothorax and phrenic nerve interruption, making a total of 27 patients. Of those in Group A, one patient with a pneumothorax had cavity closure after a phrenic nerve interruption was added. Two patients did not achieve cavity closure after phrenic nerve interruption until pneumothorax had been added. Two of the 16 patients failed to attain cavity closure with pneumothorax and phrenic nerve interruption. Thoracoplasty in both patients closed the cavity and "converted" the sputum. There was one death in the series from late bilateral spread of disease. This patient survived five years after temporary phrenic paralysis and pneumothorax had been induced. One patient whose cavity closed after phrenic nerve interruption but subsequently reopened attained closure again after pneumothorax induction. Insuffi-

cient time has elapsed since the second closure of the cavity to permit classification of her tuberculosis as arrested, but her prognosis appears to be good. Fourteen of 16 patients in Group A have inactive disease one to 168 months after sanatorium discharge (average follow-up period, 48.6 months after discharge).

TABLE 5

*Therapeutic Results in 108 Patients with Tuberculous Cavities in Lower Lobes**

	INCLUDING SUPPLEMENTARY THERAPY					EXCLUDING SUPPLEMENTARY THERAPY			
	Patients	Disease Inactive	Disease Active	Dead	Average Follow-up in Months Since Discharge	Patients	Disease Inactive	Disease Active	Dead
Pneumothorax									
Group A (unilobar disease)	15	12	2	1	67.5	13	11	1	1
Group B (multilobar disease)	16	7	2	6†	85	14	6	1	7
Total	31	19	4	7	73.5	27	17	2	8
Phrenic paralysis									
Group A	18	14	4	0	39.5	17	13	4	0
Group B	10	7	3	0	28.2	6	4	2	0
Total	28	21	7	0	35.8	23	17	6	0
Pneumothorax and phrenic paralysis									
Group A	16	14	1	1	48.6	13	11	1	1
Group B	11	8	2	1	74	7	5	1	1
Total	27	22	3	2	53.6	20	16	2	2

* In the first part of this table the final results of the initially used procedures and also of any supplementary operations needed are shown. As can be seen by comparing this with the second part of the table, the results are not significantly altered by the subtraction of the few patients who had supplementary treatment.

† One other patient died of carcinoma, his tuberculosis inactive.

In Group B, 11 patients were treated with pneumothorax and phrenic nerve interruption. In one patient, cavity closure failed to take place with pneumothorax but occurred after phrenic paralysis was added. In 4 patients, the cavity failed to close with the combined treatment. In 2 of these, thoracoplasty closed the cavity; and in one, resection was done but the patient is still hospitalized with active disease. The other patient developed an empyema and died.

Of 11 patients in Group B, 8 have inactive disease 3 to 159 months after sanatorium discharge (average follow-up period, 74 months after discharge), 2 have active disease and are hospitalized, and one died of tuberculosis (tables 3 and 5).

Thoracoplasty

Ten of the group of 103 patients had thoracoplasty over the lung which presented a lower lobe cavity on admission. Three of these patients originally had solitary lower lobe disease (Group A) and 7 had more widespread disease (Group B). All 10 patients had had unsuccessful collapse treatment prior to the thoracoplasty, 4 having had phrenic nerve interruption; 4, pneumothorax plus phrenic; and 2, pneumothorax alone.

The indications for thoracoplasty were as follows:

Open cavity	5
Open cavity plus empyema	1
Empyema with sputum positive for tubercle bacilli and questionably open cavity	3
Bronchopleurocutaneous fistula (after open cavernostomy)	1

Of the 10 patients who had thoracoplasty, 7 had inactive disease 12 to 159 months following discharge from the Sanatorium. In 2 patients the disease is still active. In one of these 2, the upper lobe and apical segment of the lower lobe were resected subsequent to the thoracoplasty. This patient now has no roentgenographic evidence of active disease, but has intermittently "positive" sputum and is coughing up sutures from the site of bronchial closure. The other patient with active disease had a cavernostomy for residual cavity (post-thoracoplasty), and now refuses resection. One of the 10 patients died of a hematogenous spread of the tuberculosis after thoracoplasty. A satisfactory thoracoplasty was not possible in this patient due to a posterior cavernostomy opening. All of the patients who had eight or more ribs removed during the thoracoplasty have inactive tuberculosis at present. One patient had seven ribs resected and a later revision with two additional ribs removed. She is the patient mentioned above who had the resection. The other 2 patients had seven-rib thoracoplasty operations performed. Thus, in these particular patients, when more than seven ribs were resected good results were obtained regarding cavity closure and arrest of the tuberculosis.

Cavernostomy

Eight of the entire series of 103 patients had cavernostomy performed for the lower lobe cavity. Five of these had closed cavernostomy of the Monaldi type and 3 had open cavernostomy. Three patients originally had unilobar disease (Group A) and 5 had more extensive disease (Group B). All but one of the 8 patients had contralateral collapse therapy sometime during the course of treatment. Seven patients had cavities more than 5 cm. in diameter; one patient had a cavity less than 3 cm. Six patients had thick-walled cavities, and 6 patients had fluid levels within the cavities.

In 5 patients the cavernostomy was unsuccessful in that it failed to close the cavity. Two of these had closed cavernostomy (Monaldi), and 3 had open cavernostomy. Two of the 5 patients with cavernostomy have inactive tuberculosis as a result of resection 2 and 21 months after discharge from the Sanatorium. One patient with a cavity under a thoracoplasty is living at home and refuses resec-

tion. Two of the patients with cavernostomy failure died, one of meningitis and one of hemorrhage. One of these 2 patients had thoracoplasty and phrenic nerve interruption as further treatment, and one had no further treatment.

Three patients attained successful closure of the cavity by cavernostomy. In all 3, the cavernostomy was of the closed or Monaldi type, complementary phrenic paralysis being used in 2 of the 3 patients. Two of these patients were readmitted with spread of disease one and one and one-half years, respectively, after discharge from the Sanatorium. Both are still hospitalized with active tuberculosis. One patient is at home on limited activity after a contralateral thoracoplasty, and has intermittently "positive" sputum. Thus, 5 of the 8 patients had a spread or exacerbation of their disease after cavernostomy. Two died of hemorrhage, 2 have inactive disease after resection, and one patient has been rehospitalized with active disease.

TABLE 6

*Results of Resection for Lower Lobe Cavities**

	NUMBER OF PATIENTS	DISEASE INACTIVE	DISEASE ACTIVE	AVERAGE FOLLOW-UP AFTER SANATORIUM DISCHARGE
Group A (unilobar disease)	8	6	2	24.3
Group B (multilobar disease	4	2	2†	14.0
Total	12	8	4	21.7

* None of the 12 patients had successful supplemental surgery.

† One of these patients is at home with intermittently positive cultures, has coughed up sutures, and is classified as arrested.

In summary: No patient now has inactive disease as a result of the cavernostomy alone; no patient now has inactive disease as a result of cavernostomy in combination with phrenic interruption or thoracoplasty; and 2 patients (the only ones of the cavernostomy group) now have inactive disease following resection, the cavernostomy having failed to close the cavity.

Resection

Twelve of the 103 patients in the series underwent pulmonary resection after the failure of other surgical procedures (table 6). The indications for resection were as follows: open cavity, 7 patients; blocked cavity, 3 patients; blocked cavity with bronchiectasis, one patient; and tuberculous bronchiectasis, one patient.

The previous treatment of the 12 patients consisted of: pneumothorax, 6 patients; phrenic nerve interruption, 2 patients; cavernostomy, 2 patients (one open and one closed); and pneumothorax plus thoracoplasty, one patient. Thus, only one patient had a resection without previous treatment of the tuberculosis.

Eight of the 12 resection patients originally had solitary unilobar disease (Group A); 3 of these had a spread of tuberculosis prior to resection. Two pa-

tients had late spread of disease after resection and are still hospitalized; they have been treated, thus far unsuccessfully, by thoracoplasty and streptomycin, respectively. Of the 8 Group A patients, 6 have inactive disease 8, 14, 22, 32, 34, and 36 months after discharge from the Sanatorium. Four of these 8 resection patients did not have streptomycin during surgery; all 4 now have inactive tuberculosis.

Of the 4 patients who had tuberculosis in more than one lobe when first seen (Group B), 2 have inactive disease 2 and 26 months following discharge from the Sanatorium. One patient had a late contralateral spread of disease while hospitalized and is still in the Sanatorium. The other patient became pregnant soon after resection, carried her baby to term, and now, two years after resection, is clinically well but still has occasional sputum cultures positive for *M. tuberculosis*, and has coughed up some silk sutures. Presumably the tubercle bacilli are coming from this bronchial source. All 4 patients in Group B had streptomycin during surgery.

TABLE 7

Over-all Results in 103 Patients with Tuberculous Cavities of Lower Lobes

	NUMBER OF PATIENTS	DISEASE INACTIVE	DISEASE ACTIVE	DEAD
Group A (unilobar disease)	57	48	4	5
Group B (multilobar disease)	46	25	8	13
Total	103	73	12	18

The complications of resection were as follows: bronchopleural fistula and empyema, one patient (now with inactive disease); and late spread of disease, 3 patients (all still hospitalized with active disease). Of the 12 patients who underwent resection, 5 had lower lobectomy, 3 had right, middle, and lower lobectomy, and 4 had segmental resection (one, apex of lower lobe; and 3, a part or all of the upper lobe with the apex of lower lobe). Of the 4 patients who had segmental resection, one had a late spread of disease, and one (mentioned above) is now coughing up sutures. None of these patients had either bronchopleural fistula or empyema.

Prognosis

The prognosis of lower lobe cavitary tuberculosis is apparently little different from the more frequently situated tuberculosis in the upper portion of the upper lobe (37). Table 7 shows the over-all results in the 103 cases. Age had little effect on the prognosis except in the younger and older groups. Thus, the percentage of patients with inactive disease in the ten- to twenty-year age group and in patients older than fifty is distinctly lower than in the age groups between. The prognosis was the same for both sexes in this series of 103 patients. As might be expected, the extent of disease was of definite prognostic significance;

in 84 per cent of patients who had solitary disease (Group A) the lesions eventually became inactive, whereas this occurred in only 54 per cent of those having more widespread disease (Group B). The size of the cavity was of significance as illustrated by the following figures taken from the 57 cases in Group A:

	PATIENTS	PER CENT OF TOTAL	LESIONS EVENTUALLY CLASSIFIED AS INACTIVE	PER CENT CLASSIFIED AS INACTIVE
Cavity 2 cm. or less	13	23	13	100
Cavity 2 to 4 cm.	31	54	27	87
Cavity 4 to 6 cm.	12	21	8	67
Cavity 6 cm. or more	1	2	0	0

The thickness of the cavity wall as estimated by inspection of roentgenograms was of less significance than expected (Group A patients):

	PATIENTS	LESIONS EVENTUALLY CLASSIFIED AS INACTIVE	PER CENT CLASSIFIED AS INACTIVE
Thin	18	15	83
Medium	27	24	89
Thick	12	9	75

The presence of a fluid level (which is apparently often indicative of bronchial disease) was of prognostic significance (Group A patients):

	PATIENTS	LESIONS EVENTUALLY CLASSIFIED AS INACTIVE	PER CENT CLASSIFIED AS INACTIVE
No fluid level	24	22	93
Fluid level	33	26	79

The presence or absence of active tuberculous bronchitis as shown by bronchoscopy was not analyzed, as less than half of the patients in this series (1937–1947) were bronchoscoped.

The location of the cavity within the lower lobe seemed to have some significance. Only 74 per cent of the patients in Group A with subapical and basal cavities eventually had inactive disease, as compared with 90 per cent of those with apical cavities.

DISCUSSION

The treatment of lower lobe cavitary tuberculosis has undergone a gradual change in the last twenty years. Originally pneumothorax was extensively used (10, 12, 29), and subsequently a combination of pneumothorax and phrenic nerve interruption was thought to be the optimal treatment (13, 21, 30) for this condition. Within the last ten years, pneumothorax has lost much of its former popularity (31) and phrenic paralysis and pneumoperitoneum in com-

bination have become increasingly popular (15, 26). Moreover, since 1944 resection has been more frequently mentioned as being effective treatment of lower lobe cavity (32, 33, 34, 35). In the present series the majority of the pneumothorax cases were treated prior to 1945 and the majority of the phrenic paralysis cases were treated since that date. All of the resections have been performed since 1945.

The results of treatment of lower lobe cavitary tuberculosis in this series indicate that pneumothorax, phrenic nerve interruption, and a combination of the two are all effective (tables 3 and 5). The slight differences in results in the three groups are not statistically significant, although it should be stated that the follow-up period in the phrenic paralysis group is somewhat shorter (table 5) and the cavities averaged less in size (table 4). These results confirm those of Crofton (37) who found pneumothorax to be effective in closing cavities in the apex of the lower lobe in 75 per cent of pneumothorax-treated patients. The results appear to extend Crofton's conclusions regarding pneumothorax to phrenic nerve interruption with or without pneumoperitoneum or phrenic belt. It is believed, therefore, that phrenic nerve interruption with complementary pneumoperitoneum should be tried in most cases before pneumothorax in the treatment of lower lobe cavitary disease, as the former treatment is simpler and probably less dangerous with regard to complications. No conclusions can be drawn from this series as to the advisability of adding pneumoperitoneum or a phrenic belt to the phrenic paralysis. However, with a single exception, either pneumoperitoneum or a belt was used in every patient with a cavity more than 3 cm. in diameter.

Since streptomycin was used in so few patients in either group, its influence cannot be evaluated. The future will disclose whether the results of resection, as determined several years after operation, will compare with the results of pneumothorax and phrenic paralysis, alone or in combination. The writer's reluctance to perform initial resection is based on the desire to allow stabilization of the lesions to take place and upon the probability that the less extensive and less dangerous procedures of phrenic paralysis and pneumothorax may be all that are necessary to render the tuberculosis inactive. Moreover, a less extensive resection may be possible if stabilization of the disease is allowed to occur.

The use of cavernostomy is probably contraindicated in lower lobe cavities except in patients whose pulmonary reserve is so reduced that little else can be done or, in rare instances, in preparation for resection. This opinion is borne out in this series of 8 patients who had cavernostomy. In none of these patients was the tuberculosis permanently arrested except in the 2 who had resection following cavernostomy. Nevertheless, a patient who presents a large chronic cavity and raises copious sputum may be a better risk for resection after a cavernostomy has decreased the amount of sputum.

Thoracoplasty over only the lower lobe area, as described by Freedlander (27), was not used in this series. Posterolateral thoracoplasty in stages from the apex down was used in patients who had empyema or active upper lobe tuberculosis as well as a lower lobe cavity. This type of thoracoplasty was quite successful in

accomplishing lower lobe cavity closure and sputum "conversion" (7 of 10 patients). It should be noted that at least eight ribs were removed in all of the successful cases. Discussion of the use of thoracoplasty in this type of mixed upper and lower lobe disease is beyond the scope of this presentation.

One of the characteristics of lower lobe pulmonary tuberculosis is said to be the high incidence of bronchial disease (26). This is borne out in the present series, since a large number of patients showed cavities with fluid level, atelectasis of a portion or all of the lower lobe, or bronchoscopic evidence of endobronchial tuberculosis. Forty-two of 57 patients (74 per cent) with solitary lower lobe disease (Group A) had at least one of these findings indicative of tuberculous bronchitis. The frequency of bronchial involvement in lower lobe tuberculosis leads to early cavity formation (13, 28), and endobronchial disease has probably contributed to the poor results obtained in other sieries (26). The high incidence of bronchial tuberculosis in this series suggests that the prognosis of lower lobe cavitary tuberculosis will improve with the increased use of streptomycin, since this drug is usually so effective in tuberculous bronchitis.

SUMMARY AND CONCLUSIONS

Among the patients with cavitary tuberculosis who entered the Michigan State Sanatorium in a ten-year period, 20 to 25 per cent had lower lobe cavitation.

A group of 103 patients with lower lobe cavitary tuberculosis has been collected for study from the Michigan State Sanatorium, Howell, Michigan, and the University Hospital, Ann Arbor, Michigan. Of these, 57 patients had tuberculosis limited to one lower lobe (Group A) and 46 had more widespread disease with the lower lobe cavity presenting the principal therapeutic problem (Group B).

Collapse therapy was used in 97 of the total group of patients; the great majority received pneumothorax or phrenic nerve interruption, alone or in combination. The results of therapy with regard to cavity closure and the patients' status observed at an average follow-up period of four and one-half years after discharge from the Sanatorium did not differ appreciably in the three groups. The results in the cases with more widespread tuberculosis were less good than in the unilobar cases, particularly when treated with pneumothorax, which showed a case mortality which approached 50 per cent.

Roentgenographic or bronchoscopic evidence of tuberculous bronchitis was present in 74 per cent of patients having tuberculosis limited to the lower lobe.

The number of patients who had thoracoplasty, cavernostomy, or resection was too small to evaluate.

The over-all results in the series of 103 patients were as follows: in 73 patients, the tuberculosis has become inactive; in 12, the disease has remained active; and in 18 patients, the tuberculosis has proved fatal.

In the 57 patients with tuberculosis limited to the lower lobe, the disease has become inactive in 48 patients; remains active in 4; and has proved fatal in 5 patients.

SUMARIO Y CONCLUSIONES

Cavernas Tuberculosas del Lóbulo Inferior; Resultados Terapéuticos en 103 Enfermos

Entre los enfermos con cavernas tuberculosas ingresados en un decenio en el Sanatorio del Estado de Míchigan, de 20 a 25 por ciento las tenían en el lóbulo inferior.

Un grupo de 103 enfermos con cavernas tuberculosas del lóbulo inferior ha sido tomado para estudio del Sanatorio del Estado de Míchigan, en Howell, y el Hospital de la Universidad, en Ann Arbor, Míchigan. De ellos, 57 tenían tuberculosis limitada a un lóbulo inferior y 46 afección más difusa, pero planteando la caverna del lóbulo inferior el principal problema terapéutico.

En 97 por ciento del grupo total, se empleó la colapsoterapia, recibiendo la inmensa mayoría neumotórax, interrupción del frénico, o una combinación de ambos. No discreparon mayor cosa los resultados de la terapéutica con respecto a cierre de cavernas y estado final del enfermo, en un período de observación que promedió cuatro años y medio después del alta del Sanatorio. En los casos con tuberculosis más difundida, los resultados fueron menos buenos que en los unilobulares, en particular los tratados con neumotórax, que mostraron una mortalidad próxima a 50 por ciento.

En 74 por ciento de los pacientes que tenían tuberculosis limitada al lóbulo inferior, había signos radiográficos o broncoscópicos de tuberculosis bronquial.

Aunque se discute el empleo de la toracoplastia, la cavernostomía y la resección, el número de enfermos así tratados es insuficiente para justipreciación.

Los resultados globales en los 103 enfermos fueron los siguientes: en 73 enfermos, inactivos, en 12 enfermos, activos, y en 18 enfermos, fallecidos.

En los 57 con tuberculosis limitada al lóbulo inferior, el desenlace fué: en 48 enfermos, inactivos, en 4 enfermos, activos, y en 5 enfermos, fallecidos.

RESUME ET CONCLUSIONS

Tuberculose cavitaire de lobe inférieur—Résultats du traitement 103 malades

Chez les malades atteints de tuberculose cavitaire ayant été hospitalisés au Michigan State Sanatorium pendant une période de dix années, 20 à 25% des cavernes étaient situées à la base.

Un groupe de 103 malades du Michigan State Sanatorium à Howell, Michigan et de University Hospital de Ann Arbor, Michigan présentant des lésions cavitaires do la base a été étudié. Chez 57 d'entre eux la tuberculose n'intéressait 'qu'une des bases (Groupe A); chez les 46 autres les lésions étaient plus étendues, les lésions cavitaires du lobe inférieur présentant le problème thérapeutique principal (Groupe B).

La collapsothérapie a été pratiquée chez 97 malades de ce groupe, la plupart étant traités par le pneumothorax, la paralysie du phrénique ou une combinaison des deux procédés. Les résultats du traitement dans les trois groupes en ce qui concerne l'oblitération des cavernes et le sort ultime du malade ne se sont pas

montrés très différents; les malades ont été suivis pendant une moyenne de quatre ans et demi après leur départ du sanatorium. Les résultats dans le cas de lésions tuberculeuses plus étendues, en particulier ceux du pneumothorax, étaient moins bons que ceux obtenus dans les lésions unilobaires; ils atteignaient un pourcentage de mortalité voisin de 50%.

Des signes radiologiques et broncoscopiques de la bronchite tuberculeuse étaient visibles chez 74% des malades atteints de lésions tuberculeuses n'intéressant que les bases.

Le nombre de malades ayant été soumis à la thoracoplastie, au drainage endocavitaire ou l'exérèse était trop réduit pour évaluation. Leur emploi est discuté.

Les résultats d'ensemble obtenus dans ces 103 cas étaient les suivants: Dans 73 malades, stabilisés; dans 12, en activité; 18 malades, décès.

Chez les 57 cas de tuberculose limitée aux bases les résultats étaient les suivants: dans 48 malades, stabilisés, dans 4, en activité, 5 malades, décès.

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THE TREATMENT OF MINIMAL PULMONARY TUBERCULOSIS CONFINED TO THE APEX OF ONE LUNG[1,2]

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INTRODUCTION

The treatment of minimal pulmonary tuberculosis is becoming more important every year. This is so because intensive case-finding methods are rapidly increasing the incidence of minimal lesions amongst the sanatorium population. Today approximately one-third of all patients on treatment at Manitoba Sanatorium have minimal disease. The incidence has risen from 3.5 per cent in 1921 to 33.8 per cent in 1949 (figure 1).

Not only are there more patients to treat for minimal tuberculosis, but new treatment measures have also appeared. The more important seem to be streptomycin and segmental resection and, in apical lesions, the use of limited thoracoplasty. At Manitoba Sanatorium, it is still believed that pneumothorax is the proper treatment for many minimal lesions, especially in the apex. An increasing number of apical lesions are being treated with limited thoracoplasty on this service, but only when selective pneumothorax cannot be obtained. Streptomycin is being used in minimal disease but not often when a good pneumothorax can be obtained. In many quarters the use of pneumothorax has fallen off or has been entirely abandoned. Bed rest, sometimes combined with streptomycin, is given first place in therapy; and in apical lesions, a primary limited thoracoplasty is advocated if progress is not satisfactory. Segmental resection in circumscribed caseous solitary lesions is being used with increasing frequency. The continuing preference for pneumothorax at Manitoba Sanatorium made it advisable to review the results obtained there with this procedure. An analysis of these results forms the basis of the present report.

Composition of Clinical Series

The series has been limited to patients with minimal lesions in the apex of one lung for several reasons:

(1) A series of lesions limited to one location permits a more precise evaluation of end results in treatment.

(2) The apex is the commonest site of tuberculous disease; hence lesions in this location represent the most frequent problem in treatment.

(3) Unilateral apical disease lends itself to collapse by limited thoracoplasty which frequently poses the question, should pneumothorax be abandoned for apical thoracoplasty?

[1] From the Manitoba Sanatorium, Ninette, Manitoba.

[2] Presented before the meeting of the Canadian Tuberculosis Association held at Vancouver in May 1950.

It is recognized that segmental resection can be applied to any area of the lung or to both lungs. It is hoped to extend this study subsequently to include consideration of this procedure and to compare the treatment of apical lesions with minimal lesions in other areas of the lung.

The material for the study was obtained through the clinical, laboratory, and roentgenologic files of Manitoba Sanatorium between the years 1928 and 1944. The year 1928 was selected as a starting point as all chest roentgenograms ob-

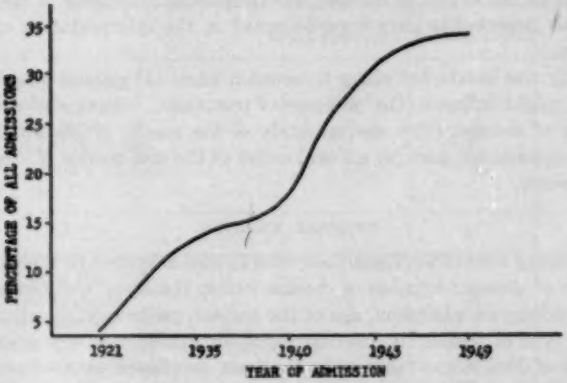

FIG. 1. Incidence of minimal tuberculosis in patients admitted to Manitoba Sanatorium.

TABLE 1

Total Patients Admitted to Hospital with Minimal Pulmonary Tuberculosis 1928–1944

DISTRIBUTION	TREATMENT 3 MONTHS OR MORE	TREATMENT LESS THAN 3 MONTHS	NO TREATMENT	TOTAL
Unilateral apical (follow-up)	112*	20	54	186
Unilateral hilar or basal (no follow-up)	46	3	7	56
Bilateral (no follow-up)	120	13	27	160
Total	278	36	88	402

* 103 of these followed for five years or more.

tained previous to this date had been discarded. In table 1 is shown the total group of patients with minimal tuberculosis who were examined roentgenographically during that period of time, together with the general distribution of lesions and the treatment intervals. In all, 402 patients had minimal tuberculosis and, of these, 278 received treatment for three months or more. Of this latter group, 112 had unilateral apical disease. The present series is confined to 103 patients in the unilateral apical group who were treated for at least three months and who had a minimum period of follow-up observation of five years from admission to the hospital.

The follow-up data were obtained through questionnaires sent to the patients and from reports of examinations at various clinics. Of the total of 103 patients, 90 per cent have been reported on since January 1949, either by questionnaire or examination, and 70 per cent have had roentgenograms since that time. As mentioned above, all have been followed for at least five years since admission, and, of these patients, 55 per cent have been followed for five to nine years and 45 per cent for ten to twenty-two years.

In regard to the extent of disease, the Diagnostic Standards of the National Tuberculosis Association have been accepted in the interpretation of minimal involvement.

The study was conducted along three main lines: (*1*) general analysis of factors which might influence the outcome of treatment, with special reference to progression of disease; (*2*) a special study of the results of pneumothorax, including complications; and (*3*) an evaluation of the end results of treatment in the entire series.

GENERAL ANALYSIS

The following factors were analyzed with special reference to their bearing on progression of disease: location of disease within the apex, sedimentation rate, bacillary findings on admission, age of the patient, pathology, duration of treatment, and type of treatment. Roentgenographic evidence of improvement or of progression of disease was taken to be the most practicable criterion on which to judge the influence of the above factors on the eventual outcome. In the following analysis, progression means a roentgenographic change indicating extension of disease or increased activity.

Relation of location of disease to progression: In the matter of location, apical is taken to mean that area of the lung above a horizontal line at the level of the second costochondral junction. Within this apical area the distribution of lesions was as follows: apical, or above the clavicle, 24; lateral subapical, 31; medial subapical, 18; combined, 30. All lesions in these various locations were found to be alike, both in behavior and end results of treatment. The original plan was to confine the study to the apical area, which is anatomically suitable for limited thoracoplasty. This was not carried out as the group was too small.

Erythrocyte sedimentation rate and progression of disease: Erythrocyte sedimentation rates were determined on 93 of the 103 patients on admission to the service. The rate was above normal in 35 and within normal limits in 58. The test did not have any prognostic significance in the present series.

Bacteriologic findings and progression of disease: In the present series no gastric washings were studied before 1932, and no cultures of gastric washings were obtained before 1941. The bacteriologic studies obtained in the 103 patients on admission to the hospital consisted of: examination of unconcentrated sputum specimens in 37 and of concentrated specimens in 2; direct examination of gastric washings in 51 patients and culture of gastric washings in 8; no examinations in 5 patients. Of those tested, the specimens obtained from 50 patients were positive for acid-fast bacilli and the specimens from the other 48 were negative.

On discharge from the hospital, the following bacteriologic studies were obtained: examination of unconcentrated sputum specimens in 28 patients and of concentrated specimens in 4; examination of gastric washings from 37; culture of gastric washings in 18; and no examination in 16 patients. In 85 of the 87 patients tested, no acid-fast bacilli were found. Thus, some form of examination of sputum

TABLE 2

Bacteriologic Studies in 103 Patients with Minimal Apical Tuberculosis

	POSITIVE FOR ACID-FAST BACILLI	NEGATIVE FOR ACID-FAST BACILLI	TOTAL
On Admission to Hospital			
Unconcentrated sputum	21	16	37
Concentrated sputum	2	0	2
Smear of gastric washings	21	30	51
Culture of gastric washings	6	2	8
Not tested			5
Total	50	48	103
On Discharge from Hospital			
Unconcentrated sputum	0	28	28
Concentrated sputum	0	4	4
Smear of gastric washings	1	36	37
Culture of gastric washings	1	17	18
Not tested			16
Total	2	85	103

TABLE 3

Relation of Bacteriologic Findings on Admission to Hospital to Subsequent Progression of Disease

BACTERIOLOGIC FINDINGS	PROGRESSION		NO PROGRESSION		TOTAL	
	Number	Per cent	Number	Per cent	Number	Per cent
Positive for acid-fast bacilli	12	24	38	76	50	100
Negative for acid-fast bacilli	15	31.2	33	68.8	48	100
Not tested	1	20	4	80	5	100
Total	28		75		103	

or gastric washings was obtained in 93.2 per cent of the patients on admission and in 83.5 per cent on discharge from the hospital.

In table 3 is shown the relation of the bacteriologic studies on admission to subsequent progression of disease. Progression of disease took place in 24 per cent of the patients who were discharging acid-fast bacilli on admission and in 31.2 per cent of the patients who were not. Thus, it would appear that those with sputum positive for acid-fast bacilli did better on treatment. This group may have

received more aggressive treatment, but the possibility seems unlikely, for pneumothorax was given to almost as many patients with negative as with positive examinations for acid-fast bacilli. The reliability of any study of the effect of "positive" sputum on the outcome of treatment depends on the thoroughness of the search for tubercle bacilli. No doubt the use of cultural methods throughout would have considerably modified the above results.

TABLE 4

Relation of Age of Patient and Nature of Lesion to Progression

AGE	PATHOLOGY	Number	PROGRESSION	
			Number	Per cent
Less than 25 years	Exudative	2	0	
	Exudative-productive	63	20	
	Productive—fibrotic	2	0	
	Total	67	20	29.8
25 years and more	Exudative	4	0	
	Exudative—productive	30	7	
	Productive—fibrotic	2	1	
	Total	36	8	22.2
All ages		103	28	27.1

TABLE 5

Relation of Length of Sanatorium Treatment to Progression of Disease

PERIODS	PROGRESSION		NO PROGRESSION		TOTAL	
	Number	Per cent	Number	Per cent	Number	Per cent
3-5 months	7	35	13	65	20	100
6-11 months	11	29.7	26	70.3	37	100
1 year and more	10	21.7	36	78.3	46	100
Total	28		75		103	

Age of patients and nature of the lesions in relation to the progression of disease: Of the 103 patients, 67 were less than twenty-five years of age and 36 were twenty-five years old and more. The lesions were divided according to roentgenographic appearance into the commonly accepted groups of exudative, exudative-productive, and productive-fibrotic. In table 4 it will be seen that 93 of the 103 lesions were in the exudative-productive group and all but one of the instances of progression occurred in this group.

Length of sanatorium treatment and progression of disease: The length of hospital treatment for the group is shown in table 5. Twenty patients were in the hospital for three to five months, 37 for six to eleven months, and 46 for one year or

more. The incidence of progression of disease was steadily less as the length of treatment increased. Thus, in the shortest treatment period, 35 per cent of the lesions progressed; in the middle period, 29.7 per cent; and in the longest treatment period, 21.7 per cent showed evidence of progression.

Type of treatment and progression of disease: The most striking factor related to progression of the disease appeared to be the type of treatment employed.

TABLE 6

Minimal Unilateral Apical Lesions

Follow-up Period 5–22 Years

Relation of Treatment to Progression

TREATMENT	NO PROGRESSION		PROGRESSION		TOTAL
	Number	Per cent	Number	Per cent	
Pneumothorax	58		15		73
Phrenic crush	7		1		8
Paraffin pack	1				1
Total with collapse	66		16	19.2	82
Rest only	9		12	57.1	21
Total	75	72.9	28	27.1	103

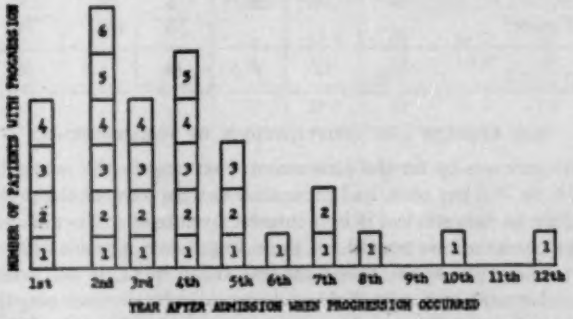

FIG. 2. Incidence of progression of minimal lesions in relation to time after admission to hospital.

As shown in table 6, of the 103 patients, 82 had some form of collapse therapy and 21 received only bed rest. Of those on rest alone, 57.1 per cent showed progression of the disease as compared to 19.2 per cent of the patients who received collapse therapy.

Incidence of progression in relation to time after admission: Figure 2 illustrates the number of patients showing progression of disease at various periods after admission. The incidence of progression was highest in the second year after admission when 6 patients had increase of disease. Of the 28 patients who showed progression, it occurred in 22, or 78.5 per cent by the end of the fifth year from

admission. However, one patient showed progression of disease twelve years after admission.

Summary of general analysis: The results of the general analysis may be summarized as follows: Factors without bearing on the progression of disease were location within the apex and the erythrocyte sedimentation rate at the time of admission to the hospital. The bacteriologic findings on admission played an uncertain role in the outcome. The incidence of progression was greater in younger age groups with exudative-productive lesions which showed the greatest instability. The incidence of progression became progressively less with longer hospital treatment. The most important single factor in reducing the incidence of progression of the disease was the use of collapse therapy.

TABLE 7

Relation of Length of Pneumothorax Treatment to Progression of Disease

PERIOD	PROGRESSION		NO PROGRESSION		TOTAL GROUP	
	Number	Per cent	Number	Per cent	Number	Per cent
Less than 1 year	3	42.8	4	57.2	7	100.0
1–2 years	5	25	15	75	20	100.0
2–3 years	4	19	17	81	21	100.0
3–4 years	1	7.7	12	92.3	13	100.0
4–5 years	2	25	6	75	8	100.0
More than 5 years			4	100	4	100.0
Total	15	20.5	58	79.5	73	100.0

THE RESULTS AND COMPLICATIONS OF PNEUMOTHORAX

Pneumothorax was by far the commonest treatment in this series. Of the 103 patients, 73, or 70.8 per cent, had pneumothorax. In view of the preference for this procedure on this service, it is of interest to note why the remaining 30, or 29.2 per cent, were not so treated. Of these 30 patients, phrenic crush was done in 3 as a primary procedure. Pneumothorax failed in 11; it was withheld in 9 because involvement was only slight and gastric cultures were negative for *M. tuberculosis;* and, in 2, the procedure was not attempted because of the patient's psychologic state. Three patients had previous or present pleural effusions and 2 refused pneumothorax. The length of pneumothorax treatment varied from four months to six and one-half years; 47 patients, or 64.4 per cent, received collapse therapy for two years or more. In table 7 will be seen the number of patients taking pneumothorax for the various time periods and the relation of these periods to progression of disease. The incidence of progression of disease dropped steadily from 42.8 per cent in those with less than a year of collapse therapy to 7.7 per cent in those with pneumothorax for three to four years. In the four- to five-year group, however, the incidence of progression of disease again advanced to 25 per cent. In general, the longer the period of collapse, the lower the incidence of progression. The pneumothorax was of short duration in many

cases. In the 7 patients in whom pneumothorax was maintained for less than one year, the procedure was terminated in 6 because of non-severable apical adhesions; and in one because of persistent pleural effusion. Of the 20 patients with pneumothorax for one to two years, the reasons for termination were as follows: duration of collapse considered adequate, 8; collapse too extensive, especially at base, 3; persistent fluid, 2; obliterative pleuritis, 2; atelectasis, 1; contralateral spread of disease, 1; sputum cultures persistently positive for *M. tuberculosis*, 1; collapse discontinued without medical advice, 2 patients.

In the 21 patients with pneumothorax for two to three years, the therapy was terminated for the following reasons: the duration of collapse was considered inadequate in 8; the collapse was too extensive, especially at base, in 9; fluid persisted in 1; obliterative pleuritis was present in 2; and hemothorax developed in one patient.

The relation of type of collapse by pneumothorax to progression of the disease is shown in table 8. In 57 patients the collapse was considered adequate and, in 16, as inadequate. Pneumonolysis was performed in 20 patients. The progression

TABLE 8

Relation of Type of Collapse by Pneumothorax to Progression of Disease

COLLAPSE	PROGRESSION		NO PROGRESSION		TOTAL GROUP	
	Number	Per cent	Number	Per cent	Number	Per cent
Adequate	9	15.8	48	84.2	57	100
Inadequate	6	37.5	10	62.5	16	100
Total	15	20.5	58	79.5	73	100

rate in patients with adequate collapse was 15.8 per cent as compared to 37.5 per cent in those with inadequate collapse.

As stated before, the most important factor in lowering the incidence of progression of disease appears to be the use of collapse treatment. Pneumothorax was the main form of collapse in this series, being used in 73 patients in contrast to phrenic operation in 8 and the use of a paraffin pack in one patient.

The complications of pneumothorax must also be considered when estimating its value as a treatment. In regard to immediate complication, there was no instance in the series of air embolus or accidental pneumothorax which required unusual measures. The incidence of fluid complicating pneumothorax was as follows: no fluid or only a small accumulation of fluid, 56 patients; moderate effusion in 13; and a large accumulation in 4 patients. Of those with moderate-sized effusion, only 3 had any systemic reaction. In the patients with extensive effusion, all had considerable systemic reaction; in 2 the fluid was clear; and in the other 2, empyema developed. Both of the latter patients were under treatment in 1930 and received oleothorax therapy successfully.

The most feared late complication of pneumothorax is retraction with interference in respiratory and cardiac function. Of the 73 patients who received

pneumothorax, 42, or 57.6 per cent, had slight or no retraction; retraction was moderate in 25, or 34.2 per cent; and marked in 6, or 8.2 per cent. It is difficult to estimate cardiac and respiratory function but only 2 patients now complain of any dyspnea. The cause of retraction is obscure, various factors such as pleural reaction, length of collapse, and atelectasis all playing a part. Probably the commonest general cause is poor management of pneumothorax, especially after leaving the hospital. In 19 patients in the present series, the collapse was too great at some period during the treatment. In most instances this occurred after the patient left the hospital.

TABLE 9

Type of Treatment and Results in Patients with Progression of Disease

RELAPSE TREATMENT	TOTAL	WELL	HOME CURE	STILL IN SANATORIUM	DEAD
Thoracoplasty	7*	4	1	1	1
Paraffin pack	1	1			
Pneumothorax	3	3			
Pneumothorax reinduced	1	1			
Contralateral pneumothorax	4	4			
Bed rest only	2	2			
Phrenic crush	1	1			
Bed rest at home	6	4	1		1
None	3	3			
Total	28	23	2	1	2

* One patient had thoracoplasty on the contralateral side.

TABLE 10

End Results of Treatment in Minimal Unilateral Apical Lesions

(Follow-up Period 5-22 Years)

STATUS	NUMBER	PER CENT
Arrested or apparently cured	94 or	91.26
Working but disease unstable	3 or	2.91
Resting at home	2 or	1.94
On treatment in sanatorium	1 or	0.97
Tuberculous deaths, pulmonary	1 or	0.97
Tuberculoma of brain	1 or	0.97
Nontuberculous deaths	1 or	0.97
Total	103 or	100

END RESULTS OF TREATMENT

As seen in the general analysis, 75, or 72.9 per cent, of the 103 patients had no progression of disease and have remained well for five to twenty-two years after admission to the hospital. Progression of disease occurred in 28, or 27.1 per cent. The further treatment of the latter group with the results obtained is shown in

table 9. Of the 7 patients with thoracoplasty, the original treatment was bed rest only in 4; pneumothorax in 2; and phrenic crush in one. A paraffin pack was used in one patient with pneumothorax. Pneumothorax was started in 3 patients who had had bed rest only; it was reinduced in one on the same side, and started on the other side in 4 with previous pneumothorax. Two patients who had had pneumothorax were finally treated with bed rest only. Phrenic crush was done on one with a previous pneumothorax. Of 6 patients with relapse which was treated by bed rest at home, 2 had had pneumothorax and 4 had had previous bed rest in the hospital. Three patients whose disease relapsed took no further treatment, but recovered. Two of these had had pneumothorax and the other had bed rest only while in the hospital.

In table 10 is shown the present status of the entire group. Of the 103 patients, the lesions of 94 are now arrested or apparently cured; 3 are working but with active disease; 2 with active disease are resting at home; one is receiving treatment in the hospital. There were 3 deaths, one from pulmonary tuberculosis, one from tuberculoma of the brain, and one death from chronic nephritis unrelated to the patient's tuberculosis.

DISCUSSION

In the management of any disease, preferences in treatment tend to move in cycles. An aggressive phase is attended by some dramatic results, but enthusiasm is soon dulled by faulty or ill-timed application. The physician then reverts to more conservative measures until some other form of aggressive therapy comes into fashion. Many procedures are thereby abandoned that have real value, if properly applied. This treatment cycle can be clearly traced in the literature on minimal pulmonary tuberculosis in the last two decades. In the nineteen thirties many authors, including Moore (1), Thomas and Davis (2), Chadwick and Evarts (3), Turner and Collins (4), advocated that pneumothorax be used in minimal lesions without delay. The literature on the subject during the last ten years is scanty, but most writers were again favoring bed rest alone. It is safe to predict that we have now entered another aggressive phase and that reports on minimal lesions treated by primary apical thoracoplasty and segmental resection will soon be published.

As stated before, most minimal lesions have been treated aggressively at Manitoba Sanatorium for the past twenty years or more. Pneumothorax has been the treatment of choice and has not been confined to those with sputum positive for tubercle bacilli. In fact, 48 per cent of those patients with pneumothorax in the present series had sputum negative for acid-fast bacilli at the time of induction. It is believed that enough patients with minimal disease will eventually show progression to warrant the early use of pneumothorax in most instances, even in the absence of positive bacteriologic findings. Pneumothorax should be avoided in endobronchial disease. It should be abandoned when collapse is inadequate or of such extent that ultimate retraction with appreciable damage to vital capacity is feared. A satisfactory collapse should not be terminated too soon and, in the absence of complications, should be maintained for approximately three years.

Within these limits the writer has found pneumothorax a satisfactory type of aggressive therapy for apical minimal lesions.

The end results of treatment in the present series have confirmed our belief in pneumothorax for minimal apical lesions. It is hard to compare these end results with those of other series because no similar study confined to one apex has been found. Also rest treatment varies a good deal in different tuberculosis hospitals. At Ninette most patients with minimal lesions have bathroom privileges at the start but do not become fully ambulant for approximately 9 months. With adequate pneumothorax, full ambulation may be reached by the sixth month. Bobrowitz (5), in a study of 220 sanatorium treatment patients with minimal disease, found no significant difference in behavior of lesions located in various parts of the lung. Only 9 patients in his series received pneumothorax and in most instances the collapse was induced only after a relapse of the disease. The remainder of the group received only bed rest. Bobrowitz found a progression rate of 42 per cent in a maximum follow-up period of five years, as compared with a rate of 27 per cent of the patients in the present series, all of whom were followed for at least five years, and 45 per cent for ten to twenty-two years. The progression rate for the patients treated with pneumothorax in the present series was 20 per cent.

The relative merits of pneumothorax, limited thoracoplasty, and segmental resection in minimal apical disease are not easy to evaluate. Granting that agressive treatment is indicated, one still has to decide on the degree of aggression. At the present time the writer uses pneumothorax in many cases where apical thoracoplasty would not be used unless the former treatment failed. It is true that retraction and reduction of vital capacity is a definite hazard in pneumothorax. This was not a common sequel in the present series, and its incidence is reduced with present-day management. Limited thoracoplasty carries with it some definite disability, has still to stand the test of time, and cannot be applied to subapical lesions as can pneumothorax. Moreover, thoracoplasty is not a reversible procedure, and reversibility may be important if disease appears in the other lung.

To date, the lobe is the smallest lung unit removed for tuberculosis on this service, so the writer has no opinion based on experience regarding segmental resection in the extirpation of isolated minimal lesions. The successful use of this operation is being reported with increasing frequency, including the removal of isolated foci in several areas of one or both lungs. It must be kept in mind, however, that sufficient time has not elapsed for a long-term appraisal of end results. The isolated circumscribed caseated lesion often called a tuberculoma responds poorly to both pneumothorax and limited thoracoplasty, and removal by segmental or even less extensive resection would seem the treatment of choice. However, the surgical risk of the operation in most hands is not negligible. Also minimal involvement is often scattered and anatomically unsuited to segmental resection. In studying surgical lung specimens, one is impressed with the many occasions in which the roentgenogram fails to demonstrate all of the diseased areas. It follows that surgical removal of an isolated pathologic unit fails more

often than suggested by roentgenographic studies to free the lung of disease. The existence of such scattered involvement, often undetected by roentgenography, is an added reason for the use of pneumothorax.

The present series of patients who were treated predominantly with pneumothorax, with arrest of disease in 91 per cent after five to twenty-two years and a death rate of less than 2 per cent, is a strong argument in favor of the continued use of this treatment in minimal apical lesions.

SUMMARY

A study is presented of 103 patients with minimal tuberculosis confined to the apex of one lung. All had hospital treatment for three months or more, and were followed for at least five years from admission. Forty-five per cent were followed from ten to twenty-two years after admission to the hospital.

An analysis was made of factors influencing the outcome of treatment, with special reference to progression of disease. Collapse therapy was the most important factor in reducing the incidence of progression. The progression rate was 57 per cent for those patients treated with bed rest alone, 19 per cent for those with collapse therapy, and 27 per cent for the entire series. Of the 103 patients, 70.8 per cent were treated with pneumothorax and, of these, approximately one-half had sputum negative for acid-fast bacilli at the time of induction. Concerning the present status, the lesions of 94 patients are arrested or apparently cured; the disease of 6 patients is still unstable; 2 patients are dead from tuberculosis, and one from nontuberculous causes. The high percentage of good results in this series is attributed to the early application of collapse treatment.

SUMARIO

El Tratamiento de la Tuberculosis Pulmonar Mínima Limitada al Vértice de Un Pulmón

Este estudio abarca 103 enfermos con tuberculosis mínima limitada al vértice de un pulmón. Todos habían recibido tratamiento sanatorial por espacio de tres meses o más, y fueron mantenidos en observación por lo menos cinco años desde su ingreso. Un 45 por ciento de ellos fueron observados de diez a veintidós años.

Los factores que afectan el resultado del tratamiento son analizados con referencia particular al avance de la dolencia. La colapsoterapia fué el factor más importante en lo tocante a disminuir la incidencia de agravación. El índice de agravación fué de 57 por ciento para los que sólo recibieron reposo; 19 por ciento para los sometidos a colapsoterapia; y 27 por ciento para toda la serie. De los 103 sujetos, 70.8 por ciento fueron tratados con neumotórax, y de éstos, aproximadamente la mitad mostraban esputo negativo al aplicar el procedimiento. En cuanto a estado actual, 94 se hallan estacionados o aparentemente curados, 6 permanecen todavía inestables, y 2 han muerto de tuberculosis y uno de causas no tuberculosas. El alto porcentaje de buenos resultados en esta serie se imputa a la aplicación temprana de la colapsoterapia.

RESUME

Traitement de la tuberculose pulmonaire "a minima" limitée à l'apex d'un seul poumon

Présentation d'une étude sur 103 malades atteints de tuberculose "a minima" limitée à la région apicale d'un seul poumon. Tous avaient été traités en sanatorium pendant trois mois, ou plus, et ils ont été suivis pendant au moins cinq ans après; 45% d'entre eux ont été suivis pendant une période de dix à vingt-deux ans.

Les facteurs qui influent sur l'issue du traitement, en particulier sur la progression de la maladie, ont été analysés. Le taux des cas évolutifs traités par le repos seul a été de 57%; avec la collapsothérapie il a été de 19% et pour la totalité de la série ce taux a été de 27%. Sur ce nombre de 103 malades, 70.8% ont été traités par le pneumothorax; la moitié d'entre eux environ n'avaient pas de bacilles dans les crachats quand le pneumothorax a été pratiqué. La situation actuelle est la suivante: 94 paraissent guéris; 6 sont encore non stabilisés; 2 ont succombé à la tuberculose, 1 est mort à la suite d'une affection non tuberculeuse. Le pourcentage élevé de résultats favorables dans cette série de cas est attribué à l'application précoce de la collapsothérapie.

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COCCIDIOIDIN SENSITIVITY ON THE ISTHMUS OF PANAMA[1]

Report of Tests Performed on One Thousand Patients

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INTRODUCTION

The fungus, *Coccidioides immitis*, has not been isolated in the Panama Canal Zone, nor have proved cases of coccidioidomycosis been encountered clinically or at autopsy in natives of this region or in transients. Coccidioidin intradermal testing in this area might provide useful information, therefore, concerning the geographic distribution of the fungus, the sensitivity and specificity of the test itself, and the problem of nonspecific or cross reactions to coccidioidin. It is the purpose of this paper to present the findings obtained in testing 1,000 patients in this region and to discuss them in terms of these factors. These data represent an extension and confirmation of an earlier study on the Pacific side of the Isthmus (1); a preliminary report on the first 500 patients has already been published (2).

MATERIALS AND METHOD

Between October 1949 and June 1950 an attempt was made to study 1,000 patients admitted consecutively to the "nonwhite" section of Colon Hospital, Cristobal, Canal Zone. Patients on the medical, surgical, obstetrical, and pediatric services were utilized. Moribund persons were excluded from the study (3), as were 24 persons who had traveled to known endemic centers for coccidioidomycosis, such as Venezuela (4), the Argentine-Uruguay region (5), and the United States. Whether antihistaminic preparations were administered prior to or during the test period (40 to 48 hours) was not taken into consideration, an omission which in all likelihood did not significantly modify the results obtained (6).

The patients studied ranged in age from less than one day to eighty-five years. All were employees of the United States Government agencies or their dependents. Many of the older patients were originally brought to the Isthmus during the construction days of the Panama Canal (1905–1913) from the West Indies, especially Jamaica, Trinidad, and Barbados. Several had emigrated from nearby Costa Rica and Colombia. The majority, however, had been born and reared on the Isthmus and had neither means nor inclination for travel. The population was a stable and a representative one, therefore, in which patients of West Indian Negro or Central American mestizo (mixed white-Indian) derivation predominated. The term "race" as applied to these categories in the following text is a loose one intended to mean a collection of individuals with similar antecedents, language, customs, and appearance.

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A complete history, physical examination, hemogram, and urinalysis were done on each patient. A 14 by 17 roentgenogram of the chest was obtained prior to discharge from the hospital. Three intradermal reactions were studied in all patients: coccidioidin, histoplasmin, and tuberculin. In addition, blastomycin and candidin were employed in representative samples of the series.

Each test consisted of the intradermal injection of 0.1 cc. of diluted antigen into the flexor surface of the forearm. All tests were performed and interpreted by the writer between forty and forty-eight hours later, using the following criteria:

Negative: no recognizable reaction; erythema only
Doubtful: induration less than 5 mm. in diameter
1-plus: induration 5–9 mm. in diameter
2-plus: induration 10–14 mm. in diameter
3-plus: induration 15 or more mm. in diameter
4-plus: vesiculation and necrosis.

Undiluted coccidioidins were provided by Dr. J. F. Kessel, University of Southern California School of Medicine (Lot A-2), and by Dr. Charles E. Smith, School of Public Health, University of California, Berkeley (Lot 29–31). These antigens were prepared from cultures of several strains of *C. immitis* grown on synthetic asparagine media (7, 8). Histoplasmins similarly prepared (9) were furnished by Dr. C. E. Roach, Medical Division, Eli Lilly Company (Lot CT-189), and by Dr. Arden Howell, Jr. of the Public Health Service (Lot H-42). Tuberculin, furnished by the Hospital, was a commercially available preparation (Parke, Davis & Company). The candidin was prepared by Dr. Joel Shrager, Board of Health Laboratory, Ancon, and the blastomycin was supplied by Dr. Howell (Lot B-7).

Histoplasmin and coccidioidin were used in a dilution of 1:100; blastomycin, in a dilution of 1:1,000; and the candidin was kept in a stock dilution of 10^{-5} for intradermal testing. Tuberculin was made up in a 1:10 dilution of second-strength purified protein derivative, the 0.1 cc. injection being equivalent to 0.0005 mg. of PPD.

At the outset, Dr. Charles E. Smith agreed to carry out specific complement-fixation and precipitin tests (5) on sera from patients reacting to coccidioidin, should such occur.

New syringes and needles were used for this study, equipment being so labeled as to prevent interchange. Prior to use, the apparatus was autoclaved. New dilutions of antigens were made every month and were kept refrigerated when not in use.

Roentgenograms were first interpreted and reported by Dr. J. W. Wilkerson, Chief of Medicine, Colon Hospital, with respect to pulmonary or cardiac pathologic findings. At the conclusion of the study all films were reviewed by the writer without reference to the results of intradermal tests, but with particular regard to evidences of single or multiple calcific lesions.

RESULTS

In the series of 1,000 patients there were 535 males and 465 females. Six hundred thirty-nine were Negroes, 256 were mestizos, and 81 had one parent of each race. There were 24 patients of "other" races, including East and San Blas Indians. Almost two-thirds of the group had never been away from the Isthmus, but 366 had traveled in Latin America or the West Indies.

As anticipated, reactors to coccidioidin were few (table 1). Pertinent data concerning these patients have been summarized in table 2. Of the 8 persons who gave positive reactions to coccidioidin, all but one were histoplasmin positive and the exception gave a doubtful response with that antigen (Case P6).

In contrast to the low prevalence of coccidioidin positivity (0.8 per cent), 61.3 per cent reacted to tuberculin, 38.7 per cent to histoplasmin, 15.8 per cent to blastomycin (in 241 patients), and 88.4 per cent to candidin (in 199 patients tested).

The small number of reactors to coccidioidin precluded generalities as to the influences of sex, race, or geographic background on sensitivity. By analogy, with the reactors to tuberculin and to histoplasmin (table 1), age may have exerted a definite effect. It was at least possible to state that, in accordance with the experience of others (10), newborn infants and children were negative regardless of the reactions of the mothers.

None of the 8 positive reactors showed evidence on physical examination or by roentgenography of recent or remote pulmonary disease. In 2 of the 8 patients who gave doubtful responses to coccidioidin, single pulmonary calcific lesions were seen (Cases D2, D7, table 2).

TABLE 1

Prevalence Ratios of Reactors Among 1,000 Patients Tested with Coccidioidin, Histoplasmin, and Tuberculin, by Ten-Year Age Groups

AGE GROUP	REACTORS TO INTRADERMAL TESTS						TOTAL NUMBER TESTED
	Coccidioidin		Histoplasmin		Tuberculin		
	Number	Per cent	Number	Per cent	Number	Per cent	
0–9			4	1.7	22	9.6	230
10–19			20	35.1	25	43.8	57
20–29	3	1.5	108	54.5	145	73.3	198
30–39	2	1.0	111	55.0	174	86.2	202
40–49	2	2.2	46	50.6	78	85.7	91
50–59			51	55.5	75	81.6	92
60–69	1	1.0	40	38.5	79	76.0	104
70–79			7	30.4	13	56.6	23
80–89					2	66.7	3
Total.....	8	—	387	—	613	—	1,000

DISCUSSION

During 1944–1945, the writer tested 154 patients with coccidioidin (Kessel's antigen; 1:100) at Gorgas Hospital, Ancon, Canal Zone (1). Using the criteria employed in the present study, there were five doubtful tests and only one positive reactor in that series, a 60-year-old Barbadian male. He has since been repatriated and his subsequent history is unknown. This incidence of reactors, 0.6 per cent, has been confirmed by the present study, the incidence for the combined series being 0.8 per cent. Although these figures are of small magnitude, they appeared to be a consistent finding in this population, at least over the span of the past five years.

How might these findings be reconciled with the apparent absence of the fungus, *C. immitis*, from the Isthmus? Were these indeed specific reactions due to sensitization of these few patients by the fungus, or did they represent cross reactions in individuals sensitized by exposure to related antigens?

The latitude of the Panama Canal Zone is approximately eight degrees north, well within the tropics, and annual rainfall is high (70 and 120 inches on the

TABLE 2

Pertinent Data Concerning Eight Positive and Eight Doubtful Reactors to Coccidioidin among 1,000 Patients Tested

	AGE	SEX	RACE	RESIDENTS OF PANAMA	INTRADERMAL TESTS						SEROLOGIC TESTS	
					Coccidioidin		Histoplasmin	Tuberculin	Blastomycin	Candidin	Complement Fixation	Precipitin
					A-2	29-31						
	years											
Positive Reactors												
P1	22	F	M	+	1+		1+	4+	1+		Negative	Negative
P2	28	M	N	+	1+		2+	2+	Negative		Negative	Negative
P3	28	F	N	+	1+	2+	2+	2+			Negative	Negative
P4	33	F	N		1+		1+	Negative	Negative		A.C.*	Cont.†
P5	35	F	N	+	1+		1+	2+	1+		A.C.	Cont.
P6	40	F	N		±	1+	±	Negative			Negative	Negative
P7	43	F	N		2+		4+	1+	1+		Negative	
P8	61	M	N		2+		2+	4+	1+		A.C.	Negative
Doubtful Tests												
D1	9 months	F	M	+		±	Negative	Negative	±	±		
D2	13	F	N	+	±		3+	Negative	1+		Negative	Negative
D3	24	M	N	+	+	Negative	2+	2+	Negative		Negative	Negative
D4	30	M	N	+	Negative	±	2+	4+		2+		
D5	45	M	M		±	±	1+	4+			Negative	Negative
D6	58	F	N		Negative	±	2+	1+				
D7	61	M	N		Negative	±	2+	4+	1+		1+,1:4;‡	
D8	67	M	N		Negative	±	2+	2+			1:8 Negative	

* Anticomplementary
† Contaminated
‡ Insignificant titer

Pacific and Atlantic sides, respectively). Precipitation is heavier during the eight months from April through November and less during the "dry season," December through March. The temperature is uniform, rarely varying from the range of 68° to 95° F. In a general way, therefore, the region resembles the southern Mississippi Basin area of the United States, in which Palmer (11) and others (12, 13) have shown the infrequency or complete absence of *C. immitis* infections of autochthonous origin. In the southwestern portion of the United States, Venezuela, and the Chaco region of Argentina-Uruguay, the areas in which the disease coccidioidomycosis is endemic are characterized by hot, windy, arid climates. It is perhaps valid to speculate that, even should infected individuals have brought the fungus to the Isthmus, the chlamydospores expectorated or otherwise originating from them would not have found a suitable environment in which to propagate and act as efficient airborne human parasites (8, 9, 14, 15). As was pointed out previously, whatever the reasons may be, *C. immitis* has not been encountered here clinically or at autopsy, and the mycologic division of the Board of Health laboratory has not isolated this fungus (16).

In the combined series, 5 of the positive reactors had lived in the West Indies and one in Costa Rica; the presence or absence of the fungus in those areas has not been determined, but they resemble the Isthmus of Panama from the standpoint of climate. The detailed geographic histories of the other 4 patients, however, showed that each had lived only in the Canal Zone and the adjacent Republic of Panama. These various considerations made it seem unlikely that these 9 reactors developed sensitivity to coccidioidin as a result of exposure to the specific fungus, *C. immitis*.

In an effort to determine whether or not cross reactions occurred with one or several of the other antigens used, the incidence and degree of reactivity to each was analyzed in terms of coccidioidin sensitivity. The high percentage of reactors to *candidin*, together with the fact that of the seven antigens employed this was the only one producing reactions in newborn (and presumably noninfected) infants, confirmed the opinion of Baer and Yanowitz (17) and others (18) that the test was of little practical value. Although superficial fungous infections, especially with *Candida albicans* and *Tinea* spp., afflict the majority of this population, had these played a dominant role in cross-sensitization to coccidioidin, one would expect an incidence of reactors significantly higher than the 0.8 per cent actually found. Furthermore, one patient with advanced pulmonary moniliasis was coccidioidin negative (Number 780).

As with the earlier series, no evidence of cross reactions with tuberculin was found; 61 patients in the combined series had bacteriologically proved tuberculosis and none reacted to coccidioidin. This was in accord with studies by Kessel (19) and by Aronson and his co-workers (20).

Analysis showed that the possibilities of cross reactions were distinctly greater in patients sensitized to the heterologous antigens, blastomycin and histoplasmin. This was in keeping with the concept of a hypothetic antigen shared by the three organisms, and even with the belief that there is a small amount only in *C. immitis*. According to Smith (13), "there seems to be a larger amount of this

hypothetical common antigen in *Blastomyces dermatitidis* and *Histoplasma capsulatum*."

Of 241 patients tested with blastomycin, 38 gave reactions. Four of these were positive and 2 gave doubtful responses to coccidioidin. Two coccidioidin reactors were blastomycin negative. In view of the small total number of coccidioidin reactors, these findings have been considered as suggestive only, but would be compatible with the work of Emmons and co-workers (9) and of Smith and his group (12).

The most convincing evidence for cross reactivity was seen in the histoplasmin-coccidioidin analysis. All coccidioidin reactors in the present series responded to histoplasmin except Case P6, in which a doubtful histoplasmin test was seen. With the exception of the nine-month-old baby (Case D1), all 8 patients who gave doubtful responses to coccidioidin showed definite intradermal sensitivity to histoplasmin. Smith and his co-workers (12) and others (9, 20–22) have published data which indicate that histoplasmin-positive patients may infrequently give cross reactions with coccidioidin. This has not been the experience of other investigators, however, in widely separated geographic areas (23–25). In the present series of 1,000 patients there were 7 four-plus reactions to histoplasmin; in only one was there a positive coccidioidin test. Of 32 who showed three-plus responses, only one reacted with a doubtful coccidioidin test. As shown in table 2, except Cases P6 and D1, the remainder of the histoplasmin reactions were equal to or greater in size than the corresponding coccidioidin results; none of the latter exceeded 14 mm. of induration in diameter. Although these data did not confirm completely the thesis (12) that the stronger the histoplasmin sensitivity, the more frequent and the larger the nonspecific reactions to coccidioidin, it appeared that the most reasonable explanation for the few reactors seen in this study was that they probably represented cross reactions in patients sensitized to histoplasmin and possibly to blastomycin as well.

The serologic tests carried out in patients reacting to coccidioidin gave further aid in interpreting the findings but only in a negative sense. In so small a group of reactors it would have been unlikely indeed to discover the disease coccidioidomycosis while antibody titers were high, as they are in the early phase of illness or in the progressive disease (5, 8, 15). The same general statement can be made concerning the roentgenographic portion of the study.

Although it was probable that the coccidioidin reactions seen in the series here reported represented cross reactions in patients sensitized to histoplasmin, to blastomycin, or to both, the basic problem remained a mystery. As mentioned in previous reports (2, 22, 26), the last recognized human case of histoplasmosis on the Isthmus was seen by Darling in 1906 (27). Except for the canine infection described by Tomlinson and Grocott in 1945 (28), *H. capsulatum* has not been found here in spite of the continued efforts of competent clinicians and pathologists. Blastomycosis, superficial or systemic, has not been recognized in this area (16). Nevertheless, intradermal testing showed prevalences of sensitivity to extracts of these fungi of 38.7 and 15.8 per cent, respectively. This discrepancy between apparent absence of specific antigens and relatively high prevalences of

intradermal sensitivity is unexplained. Since it is illogical to attempt to elucidate one baffling and unsolved problem in terms of another, it would probably be wise to conclude that the patients who reacted to coccidioidin in this series had previously been sensitized by some antigen closely related to, or an integral part of, the hypothetic antigen common to the *Coccidioides-Blastomyces-Histoplasma* group of fungi.

SUMMARY

One thousand patients indigenous to the Panama Canal Zone were tested intradermally with coccidioidin, tuberculin, and histoplasmin on the wards of Colon Hospital, Panama Canal Zone. Coccidioidin (1:100) produced positive reactions in 8 (0.8 per cent) and doubtful tests in 8 (induration less than 5 mm. in diameter). A total of 613 patients reacted to test injections of 0.0005 mg. of PPD. Histoplasmin (1:100) produced reactions in 387 (38.7 per cent), including 7 of the 8 coccidioidin-positive patients; the eighth gave a doubtful histoplasmin test. All but one patient giving doubtful reactions to coccidioidin were histoplasmin positive. Blastomycin (1:1,000) was used in 241 patients, including 6 coccidioidin reactors; 4 of these were blastomycin positive. Of 3 coccidioidin-doubtful patients tested, 2 reacted to blastomycin. Specific serologic tests on coccidioidin reactors did not yield evidence of active disease.

Coccidioides immitis has not been demonstrated in the Canal Zone and these results were interpreted as being consistent with its absence. From the data available, and in view of the apparent absence of *Histoplasma capsulatum* and *Blastomyces dermatitidis* from this region, it was believed that the few positive and doubtful reactions encountered in this series probably represented cross reactions in patients previously sensitized by some antigen closely related to, or an integral part of, the hypothetic common antigen of the *Coccidioides-Blastomyces-Histoplasma* group of fungi.

SUMARIO

Sensibilidad a la Coccidioidina en el Istmo de Panamá. Informe sobre las Pruebas Ejecutadas en Mil Enfermos

Mil enfermos indígenas de la región mencionada fueron comprobados intradérmicamente con coccidioidina, tuberculina e histoplasmina en las salas del Hospital de Colón, Zona del Canal de Panamá. La coccidioidina (1:100) produjo reacciones positivas en 8 (0.8 por ciento) y dudosas (induración de menos de 5 mm. de diámetro) en 8. Empleóse antígeno de dos procedencias distintas. Un total de 613 enfermos (61.3 por ciento) reaccionaron a inyecciones comprobadoras de 0.0005 mg. de PPD. La histoplasmina (1:100) produjo reacciones en 387 (38.7 por ciento), comprendiendo 7 de los 8 coccidioidino-positivos; el octavo acusó reacción dudosa. Todos los enfermos, menos uno, que dieron reacciones dudosas a la coccidioidina, resultaron histoplasmino-positivos. La blastomicina (1:1,000) fué usada en 241 enfermos, comprendiendo 6 reactores a la coccidioidina; 4 de éstos fueron blastomicino-positivos. De 3 con reacciones dudosas a la coccidioidina, 2 reaccionaron positivamente a la blastomicina.

Serorreacciones específicas en los reactores a la coccidioidina no rindieron datos de enfermedad activa.

El *Coccidioides immitis* no ha sido descubierto en la Zona del Canal, y estos resultados fueron aceptados como compatibles con la ausencia del mismo. A juzgar por los datos disponibles, y dada la ausencia aparente del *Histoplasma capsulatum* y el *Blastomyces dermatitidis*, de esta región, parece que las pocas reacciones positivas y dudosas observadas en esta serie representaban probablemente reacciones cruzadas en enfermos previamente sensibilizados por algún antígeno íntimamente afín, o parte integrante, del hipotético antígeno común del grupo de hongos *Coccidioides-Blastomyces-Histoplasma*.

RÉSUMÉ

Réceptivité à la coccidioidine dans la région de l'Isthme de Panama. Rapport sur les tests pratiqués sur mille malades

Mille malades, parmi les indigènes de cette région, hospitalisés à l'Hôpital de Colon, Zone du Canal de Panama, ont subi des tests intradermiques avec la coccidioidine, la tuberculine et l'histoplasmine. La coccidioidine (1%) a produit des réactions positives dans 8 cas (0,8 pour cent) et douteuses dans 8 cas (induration de diamètre inférieur à 5 mm). Des sources différentes d'antigène ont été utilisées. Un total de 613 malades ont réagi à l'injection de 0,0005 mg. de PPD. L'histoplasmine (1%) a produit des réactions dans 387 cas (38,7%), y compris 7 cas sur les 8 malades ayant une réaction positive à la coccidioidine; chez le huitième malade la réaction à l'histoplasmine était douteuse. Tous les malades, sauf un, qui présentaient des réactions douteuses à la coccidioidine avaient une réaction positive à l'histoplasmine. La blastomycine (un pour mille) a été utilisée chez 241 malades, parmi lesquels il y en avait 6 dont la réaction à la coccidioidine était positive; 4 de ces malades avaient une réaction positive à la blastomycine. Sur 3 malades dont le test à la coccidioidine était douteux, 2 ont réagi à la blastomycine. Les tests sérologiques spécifiques pratiqués dans les cas où le test à la coccidioidine était positif ne présentaient aucune manifestation d'activité de la maladie.

Le *Coccidioides immitis* n'a pas été observé dans la zone du Canal de Panama et ces résultats ont été interprétés comme étant en accord avec son absence. D'après les données disponibles et en raison de l'absence apparente de l'*Histoplasma capsulatum* et du *Blastomyces dermatitidis*, dans cette région, on a pensé que les quelques réactions positives ou douteuses observées dans ces séries d'observations, représentent probablement des réactions croisées chez des malades préalablement sensibilisés par un antigène très proche, ou faisant partie de l'antigène hypothétique commun au groupe de fungus *Coccidioides-Blastomyces-Histoplasma*.

Addendum

In March 1951, a fatal case of histoplasmosis was recognised at the Board of Health Laboratory, Ancon, Canal Zone. The victim was a three-month-old native child of West Indian ancestry from Chilibre, Republic of Panama, and the case will later be reported in detail. This case would seem to confirm nicely the opinions expressed in the preceding text.

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A SLIDE TEST MODIFICATION OF THE HEMAGGLUTINATION TEST FOR ANTIBODIES AGAINST TUBERCLE BACILLI[1]

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INTRODUCTION

Middlebrook and Dubos (1) have described a method demonstrating specific serum agglutination of erythrocytes sensitized with extracts of tubercle bacilli.[2] This has opened a new field of investigation of antibody response in animals and humans infected with tuberculosis.

This procedure, which will be referred to as the hemagglutination reaction, is performed with sensitized sheep erythrocytes. In this reaction a substance or substances (polysaccharides or protein) present in the bacillary extract is evidently adsorbed onto the erythrocytes, sensitizing them so that they agglutinate in the presence of antibodies to tubercle bacilli. Thus, it is possible to determine accurately the agglutination titer of individual sera and to follow the titer at intervals in tuberculous patients.

The method has the disadvantage of necessitating the absorption of anti-sheep cell agglutinins from each human or animal serum. It also requires long incubation and agglutination periods and cannot be completed in less than twenty-four hours. By using Group O, Rh-negative human cells, Middlebrook (2) and Gernez-Rieux (3) found the test worked equally well and the labor of absorbing the serums with sheep cells was avoided.

On becoming familiar with this hemagglutination reaction, it seemed evident that it is based on the same phenomenon as that responsible for the Coomb's Test, i.e., the detection of Rh antibodies adsorbed onto human erythrocytes by agglutination of these sensitized cells with the rabbit anti-human globulin of serum. With Coomb's method, Rh antibody is adsorbed onto the erythrocytes and it is the globulin which combines with human globulin antibodies and causes agglutination. Apparently a similar phenomenon is responsible for the hemagglutination reaction of Middlebrook, with adsorbed tubercle bacillus extracts taking the place of adsorbed Rh antibodies, and with antibodies to tubercle bacilli taking the place of anti-human globulin.

The present writers have been using in a blood bank a slide modification of the Coomb's test for Rh antibodies devised by the laboratories of the New York State Department of Health. This technique has been found to be both sensitive and accurate and to avoid nonspecific, false positive reactions.

Because of the similarity in the mechanisms of the two tests, a slide modifica-

[1] From the Division of Tuberculosis and the Division of Pathology of Grasslands Hospital and The Department of Laboratories and Research of Westchester County, Valhalla, New York.

[2] Old Tuberculin 4X International strength was kindly furnished by Dr. H. D. Piersma of Lederle Laboratories.

tion of the Middlebrook hemagglutination reaction has been developed. By using a heavy suspension of tuberculin-sensitized cells and undiluted serum, clear-cut results were obtained on the slide in a few minutes.

MATERIALS AND METHODS

The same materials were used in the slide tests as in the test tube titrations, with a few modifications.

Sera: Specimens of blood were allowed to clot firmly at room temperature, were refrigerated for several hours, and then centrifuged. Sera were stored in a deep freeze at approximately -20°C. On the day of testing, sera were thawed to room temperature and inactivated at 56°C. for thirty minutes shortly before being tested.

Cells: Human, Group O, Rh-negative cells were used, minimizing the chance of confusion from antibodies other than those against tubercle bacilli or tuberculin. This blood was taken in bottles containing acid-citrate-dextrose (ACD) solution, the anti-coagulant almost universally used in blood banks. After centrifuging, the supernatant fluid was discarded and the cells were washed three times with phosphate buffered saline (pH 7.0). On the last washing, the cells were packed for ten minutes at 2,000 r.p.m. These constituted the washed, packed cells.

Sensitization of cells: Tuberculin 4 times International Standard (Lederle) was diluted 1:12. To 48 parts of diluted tuberculin, one part of washed, packed cells was added. The cells were incubated for two hours in a water bath at 37°C. and agitated every fifteen minutes to ensure even sensitization by the tuberculin.

Preparation of 15 per cent suspension of sensitized cells: At the end of two hours, the sensitized cells were centrifuged and the supernatant fluid discarded. Care was taken not to remove any of the sedimented cells. The cells were further washed three times with buffered saline, centrifuging five minutes at 2,000 r.p.m. After the last washing, the cells were made up to volume with buffered saline to obtain a 15 per cent suspension of cells.

Preparation of 15 per cent suspension of untreated cells: A 15 per cent suspension of untreated cells was made from the washed, packed cells with buffered saline. These were used as a control with each serum tested.

Pipettes: Soft glass tubing, approximately 6 mm. inside diameter, was used to draw capillary pipettes having a final opening of approximately 1 mm. inside diameter.

The tests were set up on regular microscope slides, with a line across the center made with a wax, glass-marking pencil. Additional lines were made across each slide near each end to prevent the serum-cell mixture from running off the slide when it was tilted in performing the test.

Two small drops of undiluted inactivated serum were placed in the center of each ruled half of the slide. Six or seven slides were set up at a time. To the serum at the labeled end of the slides two drops of the suspension of sensitized cells were added and to the serum at the other end two drops of the untreated cell suspension were added. These were mixed rapidly with wooden applicators, spreading to form an oval, lengthwise of the slide, approximately 2.5 by 2 cm. in size. The slides were placed immediately on a special warmed illuminating box[3] which was tilted by hand from side to side, causing the serum-cell mixture to run from one end of the oval to the other. Results were read at the end of three minutes.

[3] Rh View Box No. 3338, American Hospital Supply Corporation.

Different percentages of cell suspensions were tried (2, 10, 15, and 20 per cent). It was found that a 15 per cent suspension was the most satisfactory. Agglutination began quickly and was usually maximal in three minutes, when the final reading was made. If allowed to remain longer on the warm box, drying begins near the edge causing a finely granular appearance which is not agglutination and should be disregarded as this might lead to recording of false positives.

In the slide test, no agglutination was interpreted as negative. Some serums caused a reaction which was suggestive of agglutination but which could not be read as definite agglutination. These were recorded as doubtful. Any degree of definite agglutination was interpreted as positive.

As the controls with untreated cells and the negative tests were tilted, the cells were seen to stream, in small currents, from one end of the drop to the other. This did not occur in the presence of even the slightest degree of agglutination. This streaming was a very good check on the presence or absence of agglutination.

In the tube agglutination reaction, titers of less than 1–8 were interpreted as *negative*, 1– 8 as *doubtful*, and titers of more than 1 – 8 as *positive*.

RESULTS

In this institution, a group of more than one hundred tuberculous patients are being followed with the original sheep cell technique, to determine if there is any correlation between agglutinin titers and the clinical status or the course of the disease in the patients, while under chemotherapy. The results of the investigation will be published at a later date.

The first slide tests were performed with tuberculin-sensitized sheep erythrocytes and properly absorbed sera. In thirteen of these sera the tests were repeated with Group O, Rh-negative, human, tuberculin-sensitized cells with identical results. Since then all slide tests have been carried out with human erythrocytes. With these same sera, titrations were also made with tuberculin-sensitized human cells and the titers were found to be the same, within the limits of experimental error, as with the sensitized sheep cells.

Blood specimens were obtained from patients in all stages of tuberculosis, from far advanced to minimal. Slide and titered hemagglutination reactions were determined on each specimen. Seventy-five sera from 57 patients were tested. The results are summarized in table 1. In this preliminary report, no attempt has been made to correlate the status of clinical activity of the patient with the results of the test.

It appears that when a titer of less than 1–8 is considered as negative, the slide test is more "sensitive" than the tube titration test. Of 20 sera from tuberculous patients giving negative titration tests, the slide test yielded 13 positive and 4 doubtful results, 17 in all. Moreover, of the 12 sera giving doubtful results with the tube hemagglutination test (titer 1–8), all gave positive slide tests.

Of the 75 sera from patients with tuberculosis, the titration test gave 57.3 per cent positive results, 16 per cent doubtful, and 26.7 per cent negative. The slide test gave 89.3 per cent positive, 6.7 per cent doubtful, and 4 per cent negative.

For a control series, specimens of blood were obtained from 31 members of

the laboratory staff. In this group, there were a few persons with long arrested tuberculosis, as would be expected to be found in any random sampling of a population, and these were included in the normal group. Table 2 shows the results of the two tests in this series.

Of the three individuals showing a positive reaction in both tests, one had had an anatomical tubercle on his thumb five years ago which healed spontaneously. He has continued to perform autopsies on fatal cases of tuberculosis. The second person has no clinical history or roentgenographic findings suggestive of tuberculosis. The third person was exposed to her tuberculous mother until the age of six years and at present has several calcified nodes in the hilar region.

TABLE 1

Correlation of Results Obtained by Slide and by Tube Agglutination Tests in Patients with Tuberculosis of Known Activity

TUBE AGGLUTINATION TEST	SLIDE TEST
Negative 20	3 Negative
	4 Doubtful
	13 Positive
Doubtful 12	12 Positive
Positive 43	1 Doubtful
	42 Positive

TABLE 2

Correlation of Results Obtained by Use of Slide and Tube Agglutination Tests on Sera from Control Series

TUBE AGGLUTINATION TEST	SLIDE TEST
Negative 18	18 Negative
Doubtful 7	4 Negative
	3 Doubtful
Positive 6	3 Doubtful
	3 Positive

Of the 31 sera in the control group, the titration test gave 19.4 per cent positive results, 22.6 per cent doubtful, and 58 per cent negative. The slide test gave 9.7 per cent positive, 19.4 per cent doubtful, and 70.9 per cent negative.

The correlation of the two types of tests is even closer in the normal group than in the group of tuberculous patients. It would seem that in a large sampling of a population, the slide test could be used to advantage in picking out negatively reacting sera. Thus, it would be unnecessary to perform the time-consuming titration test on these sera.

SUMMARY

1. A slide test modification of the hemagglutination test for antibodies to tubercle bacilli has been presented.

2. The results obtained with the slide test show a good correlation with the results obtained with the tube agglutination method, and the former is just as efficient in the detection of antibody.

3. The slide modification is faster and simpler to perform than the tube hemagglutination method.

SUMARIO

Modificación en Laminilla de la Prueba de Hemaglutinación para Anticuerpos contra los Bacilos Tuberculosos

1. Preséntase una modificación en laminilla de la prueba de hemaglutinación para anticuerpos de los bacilos tuberculosos.

2. Esta prueba guarda buena correlación con la prueba de titulación, siendo a lo menos tan delicada como ésta.

3. La modificación en laminilla es más rápida y sencilla en su ejecución.

RESUME

Un test sur lame, modification du test d'hémo-agglutination pour la recherche d'anticorps du bacille tuberculeux

1. Présentation d'un test sur lame, modification du test d'hémo-agglutination pour l'anticorps du bacille tuberculeux.

2. La corrélation entre ce test et le titrage du sérum est bonne et il est au moins aussi sensible.

3. Le test sur lame plus simple et plus rapide pourrait être utilisé avec profit comme épreuve de triage.

Acknowledgment

We wish to express our appreciation to Dr. William G. Childress and his staff for their cooperation and for furnishing us with blood samples from patients in the tuberculosis pavilion and to Dr. Gardner Middlebrook for consultation and advice while this study was in progress.

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THE IN VITRO LEUKOCYTE CYTOLYSIS TEST IN PATIENTS WITH SARCOIDOSIS[1, 2]

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INTRODUCTION

The cause of sarcoidosis is unknown. Most workers consider the tubercle bacillus to be of etiologic importance but definite proof of such a relationship is lacking. There are a few reports of the demonstration of acid-fast bacilli by examination of smears of material from sarcoid lesions (1), but the inability of the investigators to isolate the organisms by culture casts doubt on the significance of their observations. Coexistence of tuberculosis and sarcoidosis has been known to occur in a high percentage of cases (2), but the presence of other possibilities, such as a generally lowered resistance to tuberculosis in sarcoid patients, makes the interpretation of this fact difficult. The similarity of the histologic picture of sarcoidosis to certain types of tuberculosis has been mentioned as evidence for an etiologic relationship (3). Several other apparently unrelated diseases such as berylliosis and leprosy, however, produce lesions which also show a close histologic resemblance to those of sarcoidosis.

Rich and others have shown that the probable basis of the tuberculin skin reaction is injury of the sensitized cells on contact with tuberculin (4). Favour and his co-workers have shown that this cytotoxic action of tuberculin can be demonstrated on a fluid suspension of leukocytes as well as in solid tissue culture media (5).

The technique described by Favour involves the addition of a PPD solution to one of two samples of blood drawn from a tuberculous person. Normal saline is added to the second sample which is used for control observations. Immediate baseline total leukocyte counts are determined for both specimens. These counts are repeated after one hour of incubation and the results from each sample are compared with the respective pre-incubation value. A positive cytolysis test will show a fall in the leukocyte count in the specimen to which tuberculin was added but not in the control. This fall in leukocyte count is presumed to be a consequence of the specific lysis of the leukocytes from humans or animals with active tuberculosis on direct contact with tuberculin. Favour and his associates have shown that the degree of positivity of the test can be correlated with the clinical activity of the tuberculous process (6). More recently, these investigators have demonstrated that guinea pigs which have been made highly sensitive to tuberculin with killed organisms show an increased *in vitro* tuberculin leukocyte cytolysis within forty-eight hours after the production of a positive intracutaneous tuberculin reaction (7).

[1] From the Department of Dermatology and the C. Willard Robinson Foundation, Hospital of the University of Pennsylvania.

[2] This study was supported by U. S. Army Grant W-49-007-MD-473.

[3] Markle Scholar in Medical Science.

In order to study further the relationship between sarcoidosis and tuberculosis, the present writers have applied the Favour leukocyte cytolysis test to specimens obtained from a series of patients with active sarcoidosis.

METHODS AND MATERIALS

There were 17 patients in the series. All had generalized sarcoidosis, proved by examination of material obtained by biopsy. The tuberculin reaction to the intradermal injection of .005 mg. PPD was negative in all but 2 of these patients. The technique of the *in vitro* cytolysis test was carried out exactly as described by Favour (5) and his associates. Purified protein derivative (PPD) in a concentration of 50 γ per cc. was used as the test solution and normal saline was used in the controls.

RESULTS AND COMMENT

The results were as follows:

Leukocyte decrement after the addition of tuberculin to blood from patients with sarcoidosis:

Number of Patients	Median Value PPD Decrement	Median Value Saline Decrement
17	21.6 per cent (SD = 23.03)	16.9 per cent (SD = 23.92)

It is apparent that, when blood obtained from patients with sarcoidosis was studied, there was no higher rate of cytolysis in the PPD solutions than in the control preparations.

Because the significance of the leukolysis phenomenon is still not completely defined, speculation as to the significance of absence of tuberculin-induced leukolysis in a series of cases of sarcoidosis is obviously unwarranted. Hence, the results obtained in the present study should not be construed as evidence of an absence of an etiologic relationship between tuberculosis and sarcoidosis.

SUMMARY

The *in vitro* leukocyte cytolysis test has been performed on blood specimens from 17 patients with biopsy-proved generalized sarcoidosis. PPD was used as the antigen and normal saline solution for control. There was no increase in the rate of cytolysis in the samples containing PPD as compared with the controls.

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THE NATURE OF THE TUBERCULIN REACTION: FAILURE TO DEMONSTRATE IN VITRO CYTOTOXICITY OF TUBERCULIN FOR THE CELLS OF SENSITIZED ANIMALS[1]

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INTRODUCTION

In a paper which has since become a classic, Rich and Lewis (1) showed in tissue culture that the migration of leukocytes obtained from tuberculous animals was strongly inhibited in the presence of low concentrations (1:60) of tuberculin. Furthermore, such cells underwent necrosis within forty-eight hours. The killing of sensitized cells by tuberculin was considered to be an *in vitro* demonstration of the mechanism underlying the tuberculin skin reaction, and the cytotoxicity of tuberculin for sensitized cells has been widely accepted as an adequate explanation for the clinically important tuberculin test. Aronson (2, 3) confirmed the work of Rich and Lewis. In addition, this investigator differentiated the tuberculin reaction from the Arthus phenomenon by means of the *in vitro* cytotoxicity test. Subsequently, it has been shown that bone marrow, spleen, or lymph nodes may be used as the explant instead of leukocytes. Moreover, PPD has been successfully substituted for OT, and rabbits as well as guinea pigs have been used as experimental animals (4, 5).

The initial objective of the present study was to determine whether trichophytin was cytotoxic for cells obtained from guinea pigs sensitive to trichophytin (animals sensitized by infection with one of the ringworm fungi). In the course of establishing a control of the technique, however, Rich and Lewis's experiments were repeated, using tuberculin in tissue cultures of cells from tuberculous animals. The present report describes a consistent failure to confirm this well-recognized phenomenon.

METHODS AND MATERIALS

Ten large (700 gm.) white guinea pigs were the test animals. Four of these were inoculated subcutaneously with 0.5 ml. of a heavy suspension of a recently isolated strain of *M. tuberculosis*. The remaining 6 were given a similar dose intraperitoneally. The animals were tested intracutaneously with 0.005 mg. (second-strength) commercial PPD (Sharpe and Dohme) three weeks later and at regular intervals thereafter. Three intraperitoneally inoculated animals died before the skin reaction became positive. In all, 7 animals were available for the performance of the tests. Four of these were used as a source of tissue culture material at the time when the skin reactions revealed a red, indurated papule of more than 5 mm. in diameter at forty-eight hours. The remaining 3 animals showed a necrotic ulcerating forty-eight-hour papule at the skin test site at the time they were sacrificed.

In general the cutaneous reactions to tuberculin were more prominent in the animals inoculated subcutaneously than in those infected by the intraperitoneal route. Within three to five days following the elicitation of a positive skin test, the animal to be studied was

[1] From the Department of Dermatology and Syphilology, School of Medicine, University of Pennsylvania.

sacrificed by a blow on the head and tissue culture preparations were made from the bone marrow of the femur. The slide culture technique was used in all experiments.

The tissue cultures were set up as follows. One drop of chick plasma (reconstituted from Difco lyophilized material) was placed on the cover slip, followed by the bone marrow explant which was approximately 1 mm. square. One drop of PPD or OT dissolved in equal parts of human serum and Tyrodes solution was added. The human serum used in the tissue culture preparations from guinea pigs IV, V, and VII was obtained from an apparently healthy donor with a negative skin reaction to an intradermal injection of 0.1 ml. of second-strength PPD at the time of the experiment. The skin reactivity to tuberculin of the donors of serum for the other experiments was unknown. The PPD used in these preparations was in all instances the same material used to elicit the positive skin test on the corresponding guinea pig. In the control preparations, equal parts of human serum and Tyrodes solution were used as a control for the PPD cultures. The OT solution contained glycerin. The OT control cultures, therefore, were set up to contain a similar quantity of glycerin. Finally one drop of freshly prepared 10 per cent chick embryo extract was added to induce clotting. The cover slip bearing the tissue culture was sealed to a depression slide with paraffin, and incubated at 37.5°C.

Migration and cellular changes of the leukocytes were observed at four hours and appropriate intervals thereafter. Estimation of the degree of migration was made by placing the edge of the explant at the edge of the low power field and estimating the amount of migration of the cells across the field. The migrating border was not sharply circumscribed and it did not seem valid to attempt to measure the extent of migration on a more exact basis than a semi-quantitative 1 to 4 "plus." One plus represented migration across approximately one-fourth of the diameter of the field and four plus represented the entire distance. Cytologic alterations were studied in both fresh and hematoxylin-stained preparations with the oil immersion objective.

RESULTS

The results are tabulated in table 1. It is apparent that the addition of OT and PPD in extremely high concentrations did not inhibit the migration of leukocytes obtained from tuberculin-sensitive animals as compared with control preparations. The cytologic changes were minimal up to twenty-four hours in both groups. At forty-eight hours most of the leukocytes (in equal proportion from the test preparations and the controls) showed increasing vacuolization of the cytoplasm with pyknosis and degeneration of nuclei. It was impossible to detect any greater degree of cell death in the cultures containing tuberculin. Tissue cultures were performed on splenic material from 3 of these animals. The results are not tabulated, but they were similar in all respects to those obtained from the bone marrow preparations.

DISCUSSION

The results of this experiment were in complete contradiction to those of several competent investigators (1, 2, 4). The data presented here demonstrate that bone marrow explants from animals highly sensitive to tuberculin were in no way affected by the addition of high concentrations of PPD or OT *in vitro*. It should be emphasized that the details of technique of the experiments reported

here did not differ from those of the previous workers in any important respect. The animals from which the explants were taken were highly sensitive to tuber-

TABLE 1

Migration of Leukocytes in Tissue Cultures of the Bone Marrow From Tuberculin-Sensitive Guinea Pigs

PIG NUMBER	NUMBER OF TISSUE CULTURES	ANTIGENS USED AND CONCENTRATION	AVERAGE AMOUNT OF MIGRATION OF EACH TISSUE CULTURE					NATURE OF SKIN REACTION
I	4	PPD 0.005 mg/ml	++++	+++	+++	−		Red, indurated papule 6-7 mm. in diameter
	4	PPD control*	++++	++++	−	−		
II	4	PPD 0.05 mg/ml	+++	+++	+++	−		Red indurated papule 6-7 mm. in diameter
	4	PPD 0.025 mg/ml	+++	+++	+++	+++		
	4	PPD control	+++	+++	+++	+++		
III	3	PPD 0.25 mg/ml	++++	++++	++++			Red, indurated papule 8 mm. in diameter
	3	PPD 0.05 mg/ml	++++	++++	++++			
	3	PPD control	+++	+++	+++			
	3	OT 1:15	+++	+++	−			
	3	OT 1:30	++++	++	−			
	3	OT 1:60	++++	++++	++++			
	3	OT control 1:15†	+++	++	++			
	3	OT control 1:30	+++	+++	+++			
	3	OT control 1:60	+++	+++	+++			
IV	3	PPD 0.05 mg/ml	++	++	+			Red, indurated papule 6 mm. in diameter
	3	PPD control	+++	+	0			
	3	OT 1:10	+++	+++	++			
	3	1:100	++++	++++	+++			
	3	1:1,000	++++	++++	++			
	3	OT control 1:10	0	0	0			
	3	1:100	++++	++++	++			
	3	1:1,000	++++	+++	+			
V	5	PPD 0.25 mg/ml	++++	++++	++++	++	++	Ulcerated papule 10 mm. in diameter
	5	PPD 0.005 mg/ml	++++	++++	++++	++++	++++	
	5	PPD control	++++	++++	+++	+++	+++	
	5	OT 1:10	++++	++++	++++	++++	++++	
	5	OT 1:10 control	++++	++++	+++	+++	+++	
VI	5	PPD 0.025 mg/ml	++++	+++	+++	++	++	Necrotic ulcer 10 mm. in diameter
	5	PPD control	++++	++++	+++	+	+	
	5	OT 1:15	++	+	+	+	0	
	5	OT 1:10 control	+	+	+	0	0	
VII	10	PPD 0.05 mg/ml	++++	++++	+++	++	+	Necrotic ulcer 13 mm. in diameter
			++++	+++	++	+	0	
	10	PPD control	++	++	+	+	0	
			++	+	+	0	0	

* PPD control was Tyrodes solution.

† OT control was beef infusion broth containing glycerin in concentration equal to the corresponding OT solution.

culin, as shown by the strongly positive skin reactions. The OT or PPD used in the cultures in the present study was in most instances more concentrated than that of previous investigators. For example, Rich and Lewis found a marked inhibition of cell migration using 1:60 OT, whereas, in the present work, migration was not altered by OT in concentrations of 1:10. The minor details of the method, such as the type of diluting solution used, incubation temperature, and the

method of chick embryo extract preparation, were identical with those of previous investigators.

The only deviation in the present technique from that of the earlier workers was the use of human serum in the tissue cultures in place of homologous animal sera. There is no evidence to indicate that human serum will inhibit the cytotoxic action of tuberculin on sensitized cells to any greater extent than rabbit or guinea pig serum.

To the writers' knowledge, there have been no reports of unsuccessful attempts to demonstrate an *in vitro* cytotoxicity of tuberculin for leukocytes obtained from sensitized animals. Accordingly, it is appropriate to review some of the previous reports in view of the contradictions posed by the present study. Fischer found that the presence of 1:1,000 OT in fibroblast cultures from chickens with miliary tuberculosis actually stimulated the growth of these cells (6). No morphologic changes were noted. This work was confirmed by Juhasz-Schäffer, working within guinea pig spleen and kidney (7). These reports conflict with those of more recent investigators working with antigen dilutions in the same range (4). More recently Corper and his co-workers in a somewhat different type of experiment not involving tissue cultures, but dealing rather with the phagocytic activity of macrophages from tuberculin-sensitive animals, reported that OT exerted no toxic effect on the leukocytes of sensitized guinea pigs even when present in high concentrations (1 mg/ml) (8). Kirchheimer and Weiser (9) found that the desensitization of sensitized animals resulted in diminished inhibition of migration when splenic cells from such animals were grown in the presence of tuberculin. This report purports to be consistent with the findings expected from the thesis established by Rich and Lewis. On close scrutiny of the data presented for their hypersensitive control animals, however, it appears that the difference in the radius of migration between the preparations containing OT and the controls without OT was by no means striking. This criticism seems especially valid when one considers the difficulty of assigning numerical values to the radius of growth in tissue cultures in which the peripheral limit of migration forms an ill-defined zone and not a sharply demarcated line.

No explanation is at hand for the failure in the present study to confirm the widely accepted ideas of Rich and Lewis as to the cytotoxicity of tuberculin for the cells of tuberculous animals. The data have been presented simply to record a uniform failure to demonstrate such toxicity in a rather extensive series of trials. The problem obviously requires further study.

SUMMARY

Tissue cultures were performed on explants from a series of guinea pigs made tuberculin hypersensitive by infection with living tubercle bacilli. Addition of OT and PPD in high concentrations to these tissue cultures did not cause the inhibition of growth described previously by Rich and Lewis and others.

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THE SIGNIFICANCE OF RESULTS OF IN VITRO DRUG-SENSITIVITY TESTS WITH MYCOBACTERIUM TUBERCULOSIS[1, 2]

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INTRODUCTION

Determination of *in vitro* resistance to streptomycin and *p*-aminosalicylic acid of tubercle bacilli recovered from treated patients has become an essential procedure. Methods used for *in vitro* studies have received much emphasis in an effort to develop satisfactory simple techniques which would yield rapid results. The interpretation and significance of the results deserve corresponding attention. The arbitrary establishment of a definite concentration of streptomycin (usually 10 (1, 2) to 15 (3) γ per ml. of culture medium) at or above which *in vitro* growth indicates resistance has resulted in reference to patients as "sensitive" or "resistant." This practice conveys the implication, in spite of experimental observations to the contrary, that the *in vitro* test discloses a clear-cut, consistent point at which streptomycin therapy no longer benefits a patient. Inasmuch as loss of *in vitro* sensitivity to streptomycin can frequently be correlated with poor clinical response to the drug (1, 3, 4, 5, 6), though the correlation is not invariably present (1, 7), there is a tendency to regard the *in vitro* test as an unfailing guide to further treatment. It is believed that the proper interpretation of the results of drug-sensitivity tests requires considerable insight into the nature of these procedures.

The practice of planting sputum concentrates directly on drug-containing media, with proper controls (10, 11), is an improvement over earlier methods which employed ground suspensions of *M. tuberculosis* (8, 9). The direct method requires less time for decisive results in many cases (12). Moreover, the results probably reflect more accurately the sensitivity of the particular bacteria in the specimen to the drug under test, inasmuch as there is no intervening growth period which may change the proportion of resistant to sensitive bacteria existing in the sputum sample at the time of excretion by the patient. As a practical measure, the direct test has appeared to give satisfactory results in a large percentage of cases (12). It is not possible, however, to standardize the number of bacilli used in such tests. Moreover, it is not always possible to determine susceptibility of bacilli by direct testing. If the specimen contains very few tubercle bacilli, growth on non-drug-containing medium may be so meager as to render of doubtful significance lack of growth on drug-containing medium. A small number of direct tests may be contaminated with organisms other than tubercle

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[2] Published with the approval of the Chief Medical Director, Veterans Administration. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

bacilli which remained viable through the digestion procedure. Moreover, pure cultures recovered from patients are often transported elsewhere for sensitivity testing. In such instances, it becomes necessary to determine sensitivity with subcultures ("indirect" or subculture tests). In subculture testing, the inoculum can be standardized and is usually large (0.25 mg. (13), 0.1 mg. (8), or 0.01 mg. (14)). Thus, a series of observations on specimens obtained from any one patient may consist partially of results of direct tests for rapid determination of sensitivity and partially of results of indirect tests.

In a large series of tests in this laboratory, a number of discrepancies have occurred between results obtained with the direct planting method and those obtained by use of the indirect or subculture test. In other words, the excreted bacilli might be considered to be drug sensitive when the results of direct planting of sputum concentrates are used, and to be resistant when measured by subculture tests. In view of the great variation in the number of bacilli in different sputum specimens, a more thorough study of the influence of the size of the inoculum was considered essential. A tenfold variation in inoculum has been found inconsequential (8). Nevertheless, this range does not cover the large differences which may occur between the number of bacilli in a sputum concentrate and the number in the usual inoculum from a pure culture.

The following studies which have been conducted during the past two years have provided considerable information bearing on the significance of results of *in vitro* tests of drug sensitivity. They are presented with the hope that they may be of assistance in the interpretation of reports of *in vitro* studies of the susceptibility of *M. tuberculosis* to various drugs.

MATERIALS AND METHODS

Methods in current use in this laboratory for routine testing of streptomycin and PAS sensitivity were employed.

Direct tests: Equal portions of trisodium phosphate concentrates of specimens were planted on duplicate 18 or 20 mm. screw-cap tubes of 25 per cent egg yolk-beef extract agar containing 0, 1 (or 2), 10, and 100 γ of streptomycin per ml. of culture medium. Whenever pertinent, similarly prepared specimens were planted on the same medium containing, instead of streptomycin, the sodium salt of *p*-aminosalicylic acid[1] in the following concentrations: 0, 1, 10, 100, and 250 γ per ml. Results after incubation at 37° C. were recorded at weekly intervals; at four weeks they were sufficiently decisive to be considered final.

Indirect or subculture tests: Generous portions of growth from non-drug-containing medium were either ground in sterile mortars with 0.01 M phosphate buffer or placed in Tween-albumin medium for dispersed growth. Large representative samples were considered important in order to avoid errors of selection introduced if only a colony or part of a colony is used. Transfers from Tween-albumin medium were sometimes made at the end of seven to ten days' incubation to obtain more homogeneous growth and consequently better standardization of inoculum. In both cases, after preliminary periods of standing to permit settling of larger clumps of bacilli, standardization to 1 mg. per ml. was effected by the Hopkins tube method (8). Tubes of drug-containing medium with appropriate controls were then inoculated with 0.1 ml. (0.1 mg.) (8) and spread evenly over the surface of the slant. Weekly observations were made after incubation at 37° C., three-week readings being considered sufficiently decisive to be final.

[1] Generously supplied by Mr. William Steenken, Jr.

Influence of size of inoculum on result: Eighteen selected cultures of predetermined sensitivity to streptomycin or NaPAS were standardized to contain 1 mg. of bacilli per ml. Tenfold serial dilutions were made, containing from 1×10^{-1} to 1×10^{-7} mg. per ml., using separate pipettes for each dilution. One-tenth milliliter of each dilution was planted on duplicate tubes of drug-containing medium with proper controls. Final readings were made after three weeks' incubation.

Interpretation of results: Results were largely interpreted and recorded in the manner suggested by the Laboratory Subcommittee of the American Trudeau Society Committee on Research and Therapy (15). Growth on drug-containing medium was compared with growth on non-drug-containing control medium and recorded in terms of approximate per cent of control growth. If the number of colonies was sufficiently small (usually below 100), actual colony counts were made. Considerable difficulty was experienced in attempting to make accurate and reproducible estimations of growth in terms of percentage of control growth. Therefore, the validity of reading and recording results in this manner was investigated.

A detail considered essential was thorough drying of the surface of inoculated slants. Screw caps were loosened after inoculation and tubes were incubated in a horizontal position for two or three days; at the end of this period caps were thoroughly tightened to prevent drying of the medium.

RESULTS

Comparison of Results of Direct and Indirect Drug-Susceptibility Tests on Solid Medium

Pairs of drug-susceptibility tests were performed on 236 sputum specimens using (a) direct and (b) indirect methods of testing. One hundred and fifty-eight pairs of tests were run on streptomycin and dihydrostreptomycin medium and 78 pairs on NaPAS medium.

Arbitrary definitions of terms for denoting degrees of susceptibility of tubercle bacilli to streptomycin and PAS have been made with full cognizance of the fact that it has been amply demonstrated that there is no such entity as a completely susceptible culture (10, 16) and that a completely resistant culture may be equally rare. A culture was designated as drug sensitive if essentially no growth occurred in the presence of 10 γ of streptomycin per ml. or in the presence of 1 γ of NaPAS per ml. (17) during the period of observation. A culture was considered resistant if abundant growth occurred on 10 γ of streptomycin per ml. or if some growth occurred on 10 γ or more of NaPAS (17) per ml. A culture of "intermediate sensitivity" was one which produced less abundant growth on 10 γ of streptomycin than on control tubes, or some growth on 1 γ of NaPAS per ml.

The results with 236 specimens tested by both direct and subculture methods are summarized in table 1.

Using streptomycin, it was found that one of every 8 specimens (20 of a total of 158, or 12.7 per cent) would be considered sensitive to streptomycin using results of direct planting of sputum concentrates, but resistant if subculture test results were used. Furthermore, slightly more than one of every 8 cases (22 of a total of 158, or 13.9 per cent) were classified as sensitive by direct and intermediate by indirect tests, which means that direct tests in these instances failed to detect the presence of considerable numbers of resistant bacilli. Direct tests therefore failed to detect resistant bacilli in 26.6 per cent.

The number of discrepancies noted with NaPAS medium is smaller, possibly because resistance to NaPAS is less common.

The data presented under "A" in the right side of table 1 indicate that differences in size of inoculum in direct and subculture tests of the same specimen fail to account wholly for discrepancies with the two methods. The size of the inoculum, as indicated by amount of growth on non-drug-containing control tubes, also differed decidedly in a high percentage of specimens with the *same* result by the two methods of testing. For example, on streptomycin medium, inocula of markedly different size were used in the two methods in 69 per cent of 42 specimens which produced discrepant results, and in 42 per cent of 116 specimens which produced the same result. The large number of specimens with which the same result was obtained in the two tests, in spite of inocula of widely

TABLE 1

Illustrating (A) Number of Discrepancies in Results of Direct and Indirect Tests Performed on Same Specimen; and (B) Some Agreement in Results with Two Test Methods in Spite of Marked Differences in Size of Inoculum

RESULT	A TEST DRUG				B SIZE OF INOCULA IN TWO METHODS DIFFERED DECIDEDLY			
	SM or DHSM		NaPAS		SM or DHSM		NaPAS	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Same* with two methods	116	73.4	63	80.8	49	42.2	27	42.8
Different† with two methods	42	26.6	15	19.2	29	69.0	10	67.0
Total tested	158	100.0	78	100.0				

* *Same* if resistant, intermediate, or sensitive by both methods, or if intermediate by direct and resistant by subculture method.

† *Different* if sensitive by direct test and intermediate or resistant by subculture test.

different size, indicated that a more direct study of the influence of size of the inoculum was necessary.

Influence of Size of Inoculum on Results

Results with representative strains of tubercle bacilli are presented in table 2. It may be seen that in some cases (strains #486, #502, #500) dilution of inoculum did not affect the results of the drug-sensitivity tests, while in other cases (strains #418, #515, #1018) it exerted considerable influence. The strains which exhibited a high preponderance of resistant organisms, such as #500 and #502, were classified as drug resistant regardless of the extent of dilution. On planting inocula of 1×10^{-5} or 1×10^{-6} mg., these cultures were as resistant as when 1×10^{-1} or 1×10^{-2} mg. were used as inoculum. Likewise, with strains which exhibited a high preponderance of sensitive bacilli, such as #486, the effect of dilution was negligible. A "resistant" strain such as #1018, however, apparently became decreasingly resistant as the total amount of planted organisms was decreased below 1×10^{-3} mg. The use of an inoculum of 1×10^{-4} mg.

of this strain resulted in the classification of this culture as sensitive or, at most, only very slightly resistant. Other cultures which contained lower proportions of resistant to sensitive bacilli are #515 and #418. These strains would be designated as resistant to streptomycin if 1×10^{-1} mg. were employed in inoculating test medium, as "partially" to "slightly resistant" if 1×10^{-2} or 1×10^{-3} mg. were used, and as sensitive if 1×10^{-4} mg. or less were planted. Again, strains similar to #186 would be considered sensitive to NaPAS regardless of the number of bacilli used in inoculating test medium, but a strain such as P70 would be classified as increasingly sensitive with decrease in the total number of bacilli inoculated.

It is obvious, therefore, that the size of the inoculum governed the results of sensitivity tests on specimens which did not contain high proportions of either resistant or sensitive bacilli. With sufficiently large inocula, such cultures would be classified as resistant, but the use of smaller inocula may result in the classification of the same cultures as slightly resistant or even sensitive. The change from "resistant" to "sensitive" occurs when the number of resistant bacilli is reduced by dilution and the final result is a reflection of the proportion of resistant to sensitive bacilli in the specimen examined. In terms of milligrams of tubercle bacilli, with the strains observed in these studies the change occurred when the inoculum was reduced to 1×10^{-4} to 1×10^{-5} mg., inocula sufficiently sizeable to produce 1 plus to 2 plus growth on non-drug-containing medium.

It appears, therefore, that direct planting of specimen concentrates may fail to detect resistant bacilli if both of the following conditions exist: the specimens contain few tubercle bacilli, and, among those few bacilli, the ones resistant to the drug are present in low proportion ("intermediate," "partially resistant," or "slightly resistant" strains). The occurrence of few bacilli, in itself, does not necessarily result in failure of detection, as shown in tables 2 and 3.

Table 3 records a few examples chosen from daily routine sensitivity tests to illustrate the influence or lack of influence of decided differences in the size of inocula used in direct and subculture tests. Specimens with high proportions of resistant or sensitive bacilli produced the same end result regardless of variation in direct and subculture test inocula (indicated by amount of growth in control tubes). Contrariwise, specimens containing intermediate proportions of resistant to sensitive tubercle bacilli were considered "sensitive" by the direct method and "resistant" by the indirect method *as a result of* differences in the size of the inoculum (figure 1). In such tests, the number of bacilli in the specimen was sufficient to produce as much as 1 plus to 2 plus or even up to 4 plus growth in non-drug-containing control tubes of direct tests (#17, #360, #20).

Interpretation of the amount of growth: With 15 strains of *M. tuberculosis*, the growth which resulted from planting the same volume of measured inocula in tenfold dilutions varied slightly but was sufficiently consistent to permit the summary in table 4. The amounts of growth were compared by estimates made on the basis of slant coverage: (a) in terms of plusses, (b) in terms of percentage of control growth (i.e., on the tube inoculated with 0.1 mg.); and (c) in terms of the per cent growth expected according to the milligrams of bacilli inoculated.

TABLE 2

The Effect of Decreasing Numbers of Tubercle Bacilli in Inoculum on Results of Sensitivity Tests

MG. OF INOCULUM	STREPTOMYCIN											
	#486*				#418*				#515*			
	0	2	10	100†	0	2	10	100†	0	2	10	100†
1×10^{-1}	4+	100%	0	0	4+	100%	100%	0	4+	100%	100%	0
1×10^{-2}	4+	90%	0	0	4+	100%	40%	0	3+	100%	60%	0
1×10^{-3}	3+	90%	0	0	3+	100%	4%	0	3+	100%	10%	0
1×10^{-4}	1+	50%	0	0	2+	100%	12–15 c.‡	0	2+	100%	8–10 c.	0
1×10^{-5}	41–45 c.	5–7 c.	0	0	1+	100%	0	0	1+	100%	0	0
1×10^{-6}	1–3 c.	0–1 c.	0	0	13–20 c.	11–12 c.	0	0	32–56 c.	25–40 c.	0	0
1×10^{-7}	0–2 c.	0	0	0	2 c.	1 c.	0	0				
	STREPTOMYCIN											
	#1018*				#502*				#500*			
1×10^{-1}	4+	100%	100%	0	4+	100%	100%	15%	4+	100%	100%	100%
1×10^{-2}	4+	100%	95%	0	4+	100%	100%	2%	4+	100%	100%	100%
1×10^{-3}	3+	100%	100 c.	0	2+	100%	100%	0–5 c.	3+	100%	100%	100%
1×10^{-4}	2+	100%	11–30 c.	0	1+	100%	75%	0	2+	100%	100%	100%
1×10^{-5}	67 c.	43–59 c.	0	0	8–10 c.	14–16 c.	3–9 c.	0	1+	100%	100%	100%
1×10^{-6}	14 c.	1–6 c.	0	0	1 c.	0–4 c.	1 c.	0	13–19 c.	12–30 c.	8–24 c.	20 c.
1×10^{-7}	1 c.	0	0	0	0	0	0	0	1–2 c.	2–3 c.	0–7 c.	1–4 c.

	p-AMINOSALICYLIC ACID												
	S 418*					S P70*				S 188*			
	0	1.0	10	100	250†	0	1.0	10	100†	0	1.0	10	160†
1×10^{-1}	4+	100%	100%	95%	80%	4+	100%	50–60 c.	0–10 c.	4+	0	0	0
1×10^{-2}	4+	100%	90%	40%	1%	3+	100%	6 c.	0	4+	0	0	0
1×10^{-3}	3+	100%	50%	1%	4–6 c.	1+	30–40%	0	0	1–3+	0	0	0
1×10^{-4}	2+	100%	75%	2–4 c.	0–1 c.	36 c.	6 c.	0	0	22 c.	0	0	0
1×10^{-5}	1+	100%	20–30%	0	0	2 c.	0	0	0	0	0	0	0
1×10^{-6}	13–18 c.	18–24 c.	0–1 c.	0	0	0	0	0	0	0	0	0	0
1×10^{-7}													

* Strain number.

† Gamma SM or NaPAS per ml. of culture medium.

‡ c. = colonies.

TABLE 3

Illustrating the Effect of the Proportion of Resistant to Sensitive Bacilli on Comparative Direct and Indirect Test Results

METHOD	HIGH PROPORTION RESISTANT					HIGH PROPORTION SENSITIVE					INTERMEDIATE PROPORTION R:S				
	Strain	0	2	10	100*	Strain	0	2	10	100*	Strain	0	2	10	100*
Direct	470	12 c.†	8–12 c.	7–8 c.	5– 6 c.	330	16–60 c.	0	0	0	298	45–77 c.	15–24 c.	0	0
Indirect	470	4+	100%	100%	100%	330	4+	100%	0	0	298	4+	100%	25–30%	0
Direct	348	18 c.	2–12 c.	8–9 c.	2–14 c.	390	49 c.	0	0	0	319	6–31 c.	1–4 c.	0	0
Indirect	348	4+	100%	100%	100%	390	4+	50–100 c.	0	0	319	4+	100%	50–60%	0
Direct	297	1+	100%	100%	100%	407	1+	0	0	0	17	1+	100%	0	0
Indirect	297	4+	100%	100%	100%	407	4+	100%	0	0	17	4+	100%	50–60%	0
Direct	517	46 c.	30 c.	17 c.	0–8 c.	181M	1+	10–15 c.	0	0	360	2+	15–20%	0	0
Indirect	517	4+	100%	100%	100%	181M	4+	100%	0	0	360	4+	100%	70%	0
Direct	356	4 c.	3–4 c.	6–8 c.	0–6 c.	186	6 c.	0	0	0	20	4+	100%	0	0
Indirect	356	4+	100%	100%	100%	186	4+	1–6 c.	0	0	20	4+	100%	60–80%	0

* Gamma SM per ml. of culture medium.

† c. = colonies.

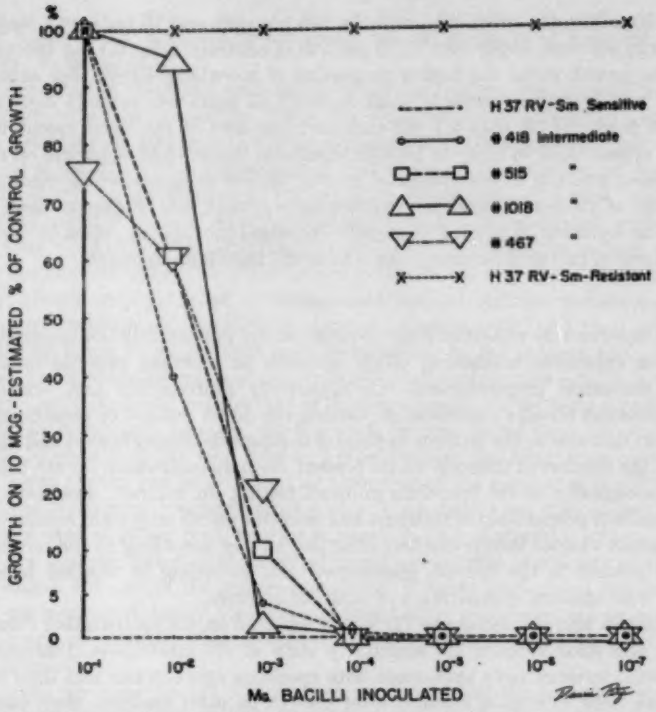

FIG. 1. Influence of number of bacilli in inoculum on interpretation of streptomycin-sensitivity tests.

TABLE 4

Illustrating Discrepancies Between Visual Estimations of Amount of Growth and Percentage Growth Expected from Size of Inoculum

INOCULUM PLANTED	ESTIMATE OF AMOUNT OF GROWTH		
	Slant Coverage	Estimated Per cent of Control Growth	
		Based on Slant Coverage	Based on Mg. Planted
Mg.			
1×10^{-1}	4+	100	100
1×10^{-2}	4+	100	10
1×10^{-3}	3+	75	1
1×10^{-4}	2+	50	0.1
1×10^{-5}	1+	25	0.01
1×10^{-6}	colonies	colonies	0.001

Inasmuch as it was not possible with 12 of 15 strains to distinguish between the amount of growth resulting from planting 1×10^{-1} and 1×10^{-2} mg., using

18- or 20-millimeter tubes (theoretically 100 per cent and 10 per cent), readings such as 25 per cent, 50 per cent, or 75 per cent of controls (using 0.1 mg. inoculum) indicate growth of far too high a proportion of inoculated bacilli. For example, 50 per cent growth in terms of slant coverage (2 plus) was actually equivalent to that produced by only 0.1 per cent to 1 per cent of the usual inoculum. It would appear that, in order to be able to express the amount of growth on drug-containing medium as percentage of growth on non-drug-containing medium or in terms of plusses, inocula for indirect tests should not be greater than 0.01 mg. The objection to the use of greatly decreased inocula is evident in the previous section on the influence of the size of the inoculum on results.

DISCUSSION

The observations presented have obvious clinical implications. Drug-sensitivity tests on specimens containing either sensitive or resistant tubercle bacilli in great numerical preponderance are apparently reproducible and consistent. This situation obtains regardless of whether the direct method of planting specimen concentrates or the indirect method of testing subcultures is used and regardless of the number of tubercle bacilli present, with due allowance for the possible non-homogeneity of the inoculum in direct testing. In contrast, specimens with intermediate proportions of resistant and sensitive bacilli may yield results which will permit various interpretations from day to day according to the number of bacilli present in the sputum concentrate and according to whether tests are made with sputum concentrates or with subcultures.

Bernstein and his associates (21) have reported on the comparative values of liquid and solid medium for sensitivity tests of *M. tuberculosis*. Inasmuch as their solid medium tests were made with specimen concentrates and their liquid medium tests with pure cultures after growth on solid medium, their comparison was rather of direct and subculture testing, with the added variable of differing media in the two types of tests. It is interesting, however, that their results were similar to those described in this report. They also found close agreement between results with the two types of tests when cultures were primarily either sensitive or resistant, with 30 per cent disagreement if the specimen tested contained "a mixture of sensitive and resistant organisms."

Each type of test has drawbacks. In the direct test, tubercle bacilli are growing on artificial medium for the first time. This handicap, added to the presence of streptomycin, probably contributes to the difficulty of detection of small numbers of resistant bacilli in direct testing. On the other hand, subculturing for the indirect test may disturb somewhat the proportion of resistant to sensitive bacilli. This disturbance is difficult to measure in view of the influence of the size of the inoculum on results with specimens containing intermediate proportions of resistant and sensitive bacilli.

Two factors, both concerned with the number of bacilli planted, exert opposing influences on results of sensitivity tests. On the one hand, the present studies have shown that the inoculum cannot be too large or an accurate differentiation of growth on drug-containing medium between 1 per cent and 100 per cent of control growth cannot be attained using the usual size of test tube; on the

other hand, if the inoculum is too small, limited numbers of resistant bacilli may not be detected.

The manner of recording estimations of amounts of growth on solid medium is important in this connection. Using the usual 18- or 20-millimeter test tube, large discrepancies may occur between estimations of the amount of slant coverage and actual maximum percentage of growth expected according to the number of tubercle bacilli inoculated. For example, an estimate of 3-plus (not quite confluent) growth is made on visual inspection of a tube inoculated with 0.001 mg. (only 1 per cent of the frequently used 0.1 mg. inoculum for indirect tests). The clinician who receives a report of 3-plus growth on 10 γ of streptomycin per ml. of culture medium is led to believe that bacilli from that patient are predominantly resistant (if growth on the control tube is recorded as 4 plus), whereas actually as many as 99 per cent may be sensitive. In the indirect test, in particular, an excessive inoculum confined to a limited surface area results in a false appearance of increased proportions of resistant bacilli. It has some significance also in the direct test, however, for if growth on the control tube is recorded as 4 plus and growth on the 10 γ tube as 2 plus, the clinician again may be misled, since 2-plus growth can be produced by 1 per cent of the number of bacilli necessary to produce growth considered as 4 plus. Thus, only 1 per cent of inoculated bacilli, instead of 50 per cent, may be resistant to 10 γ of streptomycin in such a case. The limiting factor in accuracy of visual estimations of amount of growth is, of course, the area of the surface inoculated. If larger surfaces of culture medium are inoculated, larger-sized inocula might allow more accurate estimations of amounts of growth. This procedure, however, would make an even more cumbersome test.

Previous investigators (13, 18) have attempted to find the minimum number of streptomycin-resistant tubercle bacilli necessary to classify a strain as resistant by planting artificial mixtures of known concentrations of "resistant" and "sensitive" strains on drug-containing medium. In view of the data presented in this report, it seems that results of such studies would depend entirely on the particular strains used for making artificial mixtures. If so-called "completely sensitive" and "completely resistant" stock strains are used, the result of the sensitivity test will be resistant as long as the actual number of resistant bacilli in the inoculum is sufficient to initiate growth. In other words, one is merely diluting resistant bacilli with a suspension of sensitive bacilli in such a case, in a manner similar to that used in the present studies to determine the effect of the size of inoculum using a stock resistant strain. Results of brief studies in this laboratory with artificial mixtures of strains of stock H37Rv-streptomycin-sensitive and H37Rv-streptomycin-resistant organisms appeared to bear out this interpretation.

It is known that the time permitted for growth is an important factor in determining recovery of resistant bacilli as well as in the degree of their resistance. The time of incubation in the present studies was kept constant because interest was centered on interpretation of practical results of standardized test methods as reported to the clinician. For practical purposes, the period of incubation is usually as short as is consistent with decisive results and is kept constant, es-

pecially for strains which grow only poorly or not at all on drug-containing medium.

Yegian and Vanderlinde in 1948 (19) pointed out that many factors can influence results of drug-sensitivity tests, specifying errors in sampling, length of period of incubation, characteristics of individual bacterial populations, multiplication of bacterial cells, and the fact that the drug-sensitivity test is devised to detect resistance, i.e., that there is no selective medium for detection of sensitive cells. They also have furnished evidence that the incidence of resistant variants increases as the size of the population increases, with the drug concentration remaining constant. In a later article (1950), Yegian and Vanderlinde (20) state that the clinical sensitivity test can be misinterpreted, that it is not an exact scientific tool or an accurate quantitative test by which chemotherapy (particularly with streptomycin) can or cannot be expected to benefit a patient. They also state that only when the proportion of resistant to sensitive bacilli is very high or very low is it possible for sensitivity tests to provide the physician with information on the exact sensitivity status of the invading bacilli. Confirmatory data for this viewpoint are presented in the present report. Full concurrence is expressed with the opinion of Yegian and Vanderlinde that the value of a sensitivity test is not quantitative but lies rather in an appreciation of its limitations.

Treatment with streptomycin is theoretically of benefit to a patient as long as he harbors tubercle bacilli susceptible to the action of the drug. The lack of homogeneity of cultures recovered from treated patients with respect to their sensitivity to streptomycin raises a question regarding the sensitivity studies used as bases for conclusions that treatment ceases to benefit a patient if *in vitro* tests disclose resistant bacilli. Patients are sometimes loosely referred to as "resistant" on the basis of *in vitro* tests of the organisms they discharge. At what point is the bacterial population as a whole so resistant that the effectiveness of the drug is lost? Will a small number of organisms resistant to 10 γ result in lack of response to treatment; will intermediate proportions of resistant bacilli produce such an occurrence; or should treatment be stopped only when there is a high preponderance of resistant bacilli? Attempts to answer these questions emphasize the importance of the manner of interpreting the results of sensitivity tests.

SUMMARY AND CONCLUSIONS

1. Drug-sensitivity tests made by (a) planting specimen concentrates on drug-containing medium, and (b) testing subcultures from the same specimen yielded discrepant results with 26 per cent of specimens tested.

2. The simultaneous occurrence of two conditions is responsible for these discrepancies: (a) a mixture of sensitive and resistant bacilli in the specimen with neither variant occurring in marked preponderance; and (b) marked differences in the size of inocula in the two types of tests. Specimens containing intermediate proportions of resistant to sensitive bacilli may therefore result in interpretations which will vary from day to day on the same patient, according to the output of tubercle bacilli.

3. Results of sensitivity tests of tubercle bacilli to streptomycin and NaPAS are reproducible and consistent, regardless of the number of organisms in the specimen, only if the proportion of resistant to sensitive variants is either very high or very low.

4. Current methods of reading and recording results of indirect tests convey the impression of higher numbers of resistant bacilli than actually occur in the specimen.

5. Drug-sensitivity tests cannot be used at present as unfailing guides to further treatment. Rather, they should be accepted as merely providing additional information about treated patients, to be considered with all other data in determining advisability of further treatment or change in treatment.

SUMARIO Y CONCLUSIONES

La Significación de los Resultados de las Pruebas de la Farmacosensibilidad in Vitro con el Mycobacterium Tuberculosis

1. Las pruebas de farmacosensibilidad ejecutadas: (a) sembrando ejemplares concentrados en medio que contenía droga y (b) comprobando subcultivos del mismo ejemplar dieron resultados discordantes en 26 por ciento de los ejemplares comprobados.

2. La ocurrencia simultánea de dos condiciones es la causa de dichas discrepancias: (a) mezcla de bacilos sensibles y resistentes en el ejemplar sin que ninguna de las variantes alcance mayor predominio; y (b) pronunciadas diferencias en el tamaño de los inóculos en las dos clases de pruebas. Ejemplares que contienen proporciones intermedias de bacilos resistentes y sensibles pueden, pues, dar lugar a interpretaciones que variarán de día en día en el mismo enfermo, conforme a la producción de bacilos tuberculosos.

3. Los resultados de las pruebas de la sensibilidad de los bacilos tuberculosos a la estreptomicina y al NaPAS son reproducibles y constantes, independientemente del número de microbios en el ejemplar, únicamente si la proporción de variantes resistentes y sensibles es bien altísima o bajísima.

4. Las actuales técnicas de lectura y registro de los resultados de pruebas indirectas dejan la impresión de que las cantidades de bacilos resistentes son mayores que las contenidas realmente en el ejemplar.

5. Las pruebas de farmacosensibilidad no pueden ser usadas por ahora como guías infalibles del tratamiento ulterior. Deben más bien ser aceptadas como fuente de nuevos datos acerca de los sujetos tratados, que serán considerados con todos los demás datos al determinar la conveniencia de seguir o variar el tratamiento.

RESUME ET CONCLUSIONS

La valeur des résultats des tests de sensibilité aux médicaments du Mycobacterium tuberculosis in vitro

1. Les tests de sensibilité aux médicaments ont été faits: a) en ensemencant le spécimen concentré dans un milieu contenant le produit intéressé; b) les tests practiqués avec d'autres cultures du même spécimen, ont donné des résultats contradictoires dans 26% des spécimens soumis aux tests.

2. Ces contradictions résultent de la production simultanée de deux conditions: (a) le mélange dans le spécimen de bacilles sensibles et résistants, sans prédominance de l'un ou de l'autre type de bacille; b) des différences marquées dans le volume des produits d'inoculation dans les deux types de tests. Les spécimens contenant des proportions intermédiaires de bacilles allant du type résistant au type sensible peuvent donc donner lieu à des interprétations variant de jour en jour pour un même malade selon la pullulation des bacilles tuberculeux.

3. Les résultats des tests de sensibilité du bacille de Koch à la streptomycine et au NaPAS sont reproductibles et concordants quel que soit le nombre de bacilles contenus dans le spécimen, à condition que la proportion entre les types résistants et les types sensibles soit ou très élevée ou très basse.

4. Les méthodes actuelles de lecture et d'enregistrement des résultats des tests indirects donnent l'impression qu'il y a un nombre de bacilles résistants supérieur à celui qui existe en réalité dans le spécimen.

5. Les tests de sensibilité aux médicaments ne peuvent être utilisés actuellement comme des guides infaillibles pour la poursuite du traitement. Il faut plutôt les considérer simplement comme des informations additionnelles sur les malades en traitement, informations qui doivent entrer en ligne de compte avec les autres données pour déterminer l'opportunité de la poursuite du traitement ou d'une modification dans le traitement.

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ON THE MECHANISM OF LIQUEFACTION OF TUBERCLES[1,2,3]

I. The Behavior of Endocellular Proteinases in Tubercles Developing in the Lungs of Rabbits

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INTRODUCTION

In a recent review of the pathogenetic concepts of tuberculosis, Medlar (1) concluded, "In man it is evident that the phenomenon of necrosis with subsequent liquefaction and sloughing of the necrotic areas represents the major problem in the dissemination and in the complete eradication of the disease in the person affected." Unfortunately, little is known concerning the intimate mechanism of either caseation or softening. As Lurie (2) states, "The mechanism of softening, which is the most important fact in the mortality of human tuberculosis, is unknown."

Earlier workers who investigated this problem were handicapped by inadequate methods for producing the infection and for studying the chemistry of the processes involved. Thus, in 1908, Opie and Barker (3) injected virulent tubercle bacilli into the pleural cavities of dogs and utilized the caseous material which developed in the mediastinum and in the hypertrophied regional lymph nodes. Since dogs are not generally susceptible to natural infection with tubercle bacilli and since injection into the pleural cavity is a highly artificial method for reproducing the disease, it seemed desirable to make use of the newly developed Wells apparatus for quantitative airborne infection (4).

As shown by Lurie and associates (5), Lurie and Abramson (6), Wells, Ratcliffe, and Crumb (7), and Ratcliffe and Wells (8), the Wells apparatus offers the following advantages:

(a) In contrast to methods previously employed (9, 10), which induce a diffuse caseous pneumonia and seldom produce discrete or softened tubercles, the Wells apparatus permits the development of a reproducible pattern of disease which is essentially similar in character to that acquired by natural airborne contagion.

(b) It provides for a natural portal of entry, the animals being exposed simultaneously in groups to inhalation of aerosols containing predetermined numbers of virulent bacilli in droplet nuclei.

(c) The microorganisms reach all parts of the lungs and become implanted on the alveolar walls as individual cells.

(d) The tubercles which are thus induced develop slowly and at a highly uniform rate for a considerable period of time.

(e) By causing an infection of low initial intensity, it is possible to produce isolated, discrete tubercles in visceral organs, especially in the lungs, kidneys, and spleen.

[1] From the Research Laboratories of the Jewish Hospital of Philadelphia, Pennsylvania.

[2] This study was made possible by grants from the Committee on Medical Research and Therapy of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Preliminary abstracts have appeared in *Federation Proceedings*, 1950, *9*, 348 and 394.

(f) By killing the animals at different intervals of time after infection, tubercles are obtained which show various stages of caseation and softening and which can be dissected out and subjected separately to chemical and histologic studies.

In regard to the mechanism of liquefaction of caseous tissue, it is generally assumed on the basis of the work of previous investigators (11) that this is accomplished by the autolytic or endocellular proteolytic enzymes of inflammatory cells which have arrived in response to infection. Thus, Opie and Barker (3) in 1908 studied the leukoprotease and lymphoprotease activity of hypertrophied caseous lymph nodes which they had obtained from infected dogs, employing 50 per cent heated dog- and ox-serum as substrates. After an extensive critical review of the literature, Rich (12) summarizes the knowledge of the subject as follows:

> At present it remains a problem whether the failure of autolysis to occur in caseous areas is due to an inadequate content of proteolytic enzymes in the monocytes or to the presence of substances that inhibit or destroy the enzymes at the site.

The techniques available in 1908, as well as those employed in 1914 by Jobling and Petersen (13), have been greatly improved. Accordingly, the present writers have adopted the newer enzymologic procedures and principles developed by Bergmann, Fruton, Irving, Smith, and Driscoll (14, 15). These methods permit the study of the kinetics of proteinases, the identity of which is characterized by the specific synthetic substrates which they hydrolyze.

Two proteinases, benzoyl-l-arginine amidase[4] (*BA-amidase* or Cathepsin II) and leucine-amino peptidase[4] (*LA-peptidase*) were selected for this investigation, since they are endocellular enzymes which are widely distributed throughout the body. The former (BA-amidase) is an endo-peptidase which is probably concerned in the processes of autolysis and of protein synthesis (16). It can hydrolyze central peptide linkages which are not adjacent to a free terminal amino or carboxyl group. The latter proteinase (LA-peptidase) has its origin in lymphocytes and is universally distributed in the tissues. It is an exo-peptidase requiring a substrate possessing a free amino group not necessarily adjacent to a sensitive peptide bond.

METHODS AND MATERIALS

A series of unselected, female, albino rabbits, weighing 2.5 to 3.0 kg., were used in these experiments. They were exposed to standardized aerosol suspensions of virulent bovine tubercle bacilli of the Ravenel strain, as described by Ratcliffe and Wells (8), and were killed by exsanguination from the carotid artery at intervals of seven, twelve, and twenty weeks after infection. The organs were removed, the tubercles carefully dissected out, freed of surrounding lung tissue, and immediately transferred to a dry-ice refrigerator for future study. Whenever it was desired to carry out enzymologic analyses, bits of tissue were removed from various parts of the isolated tubercles and from other areas of infected lungs. These were preserved, each separately, in Bouin's solution for histologic study. Contiguous bits of tissue, as nearly identical to the above as possible, were ground, each separately, in a Potter homogenizer (which was immersed in an ice bath) and used as source

[4] Hereinafter designated BAA and LA, respectively.

of enzyme. In this manner, comparable histologic and enzymologic data were obtained on the contents and "walls" of softened or liquefied tubercles, the centers of caseous tubercles, and the zones of inflammation surrounding them, as well as from areas of lung not primarily involved.

The substrates BAA and LA were employed in concentrations of 0.05 mM per ml. of test solution. BA-amidase activity was measured by the method of Irving, Fruton, and Bergmann (14) at pH 5.0 to 5.2 in the presence of 0.02 M citrate buffer containing 0.02 mM cysteine per ml. of test solution. LA-peptidase activity was studied at pH 7.8 to 8.4 employing 0.04 M veronal buffer in the presence of 0.001 M manganese sulfate. The rates of hydrolysis were determined by the method of Grassmann and Heyde (17) which measures the liberation of carboxyl groups. Further analytical details have been described in previous publications (9, 10).

RESULTS

Pathologic Findings

A description of the development of tubercles in rabbits exposed under identical conditions to quantitative airborne infection has been given by Ratcliffe and Wells (8). Since in the present study these results were reproduced, it will not be necessary to report the data in detail. In brief, Ratcliffe and Wells showed that it is possible to induce the development of tubercles up to diameters of 5 to 7 ml. within six weeks after infection with virulent bovine tubercle bacilli. After the sixth to the tenth week, the tubercles slowly enlarge, undergo caseation, and begin to soften. Within approximately twelve weeks after infection, the tubercles grow to diameters of 2 to 3 cm. Many of them become fused and undergo liquefaction (4).

A series of 30 infected rabbits showed positive tuberculin skin reactions when tested five or six weeks after exposure. The average size of these skin lesions was 18 by 15 by 7 mm. In animals which were killed seven weeks after infection, the lungs were studded with discrete tubercles, each approximately 6 mm. in diameter. The total number of tubercles varied from 10 to 50, the average being approximately 20. These tubercles, protruding 5 or more mm. above the pleural surface, with their central areas of caseation and their dense inflammatory walls resembled a jewel in its setting. Small secondary tubercles were found in several of the animals. The intervening lung tissue appeared grossly normal.

Histologic examination revealed varying degrees of caseation, intermingled with or surrounded by zones of dense cellular infiltration or of normal lung tissue. Of the inflammatory cells, most were monocytes and epithelioid cells, although lymphocytes and polymorphonuclear cells were also seen. A few of the tubercles had undergone softening. Grossly and microscopically, the spleens and livers were normal, but an occasional kidney showed a few tubercles which were visible to the naked eye.

In animals which were killed twelve to sixteen weeks after infection, the lungs had increased to approximately four times the normal size and weight. In addition to a varying number of primary tubercles, some of which had become fused, there were numerous small, scattered, secondary tubercles. The larger tubercles had grown to the size of 2 cm. or more in diameter and had undergone softening. Relatively little normal lung tissue remained. The retrosternal lymph nodes

were enlarged and filled with tubercle bacilli. Several small tubercles were seen through the capsules of the kidneys. The spleens and livers were not grossly involved.

In animals which were killed twenty weeks after infection, the tubercles had become fused together and had undergone softening. The secondary tubercles were too numerous to count. There were a variable number of tubercles in the cortices of the kidneys, but none in the spleens or livers.

TABLE 1

Summary: Hydrolysis of Benzoyl-l-Arginine Amidase (BAA) and Leucine-Amino Peptidase (LA) by Homogenates of Various Portions of Tubercles

PORTIONS OF TUBERCLES REMOVED FROM LUNGS OF INFECTED RABBITS	INTERVALS AFTER INFECTION AT WHICH ANIMALS WERE KILLED					
	7 Weeks		12 to 16 Weeks		20 Weeks	
	C°_{LA}	$C_{BAA} \times 10^{-4}$	C°_{LA}	$C_{BAA} \times 10^{-4}$	C°_{LA}	$C_{BAA} \times 10^{-4}$
a) Areas not primarily involved	2 N	1–1.5 N	1–2 N	1–2.5 N	1.5 N	1–1.5 N
b) Inflammatory zones	3–4 N	2–4 N	3–4 N	2.5–5 N	Wall of S.T. 2–4 N	Wall of S.T. 2–4 N
c) Caseous centers	1–2 N	1–2 N	0.3–0.5 N	0.5–0.1 N		
d) Softened (liquefied) material			0.3–0.14 N	0	0.6–0.14 N	0

K = reaction velocity constant

$K_{BAA} = \frac{1}{t} \log \frac{a}{a - x}$

a = 100 per cent hydrolysis

x = per cent hydrolysis

t = time (minutes)

C = proteolytic coefficient = K/mg. N/ml. homogenate

K°_{LA} = per cent hydrolysis/time (minutes)

N = times the corresponding values for normal lung tissue

S.T. = softened tubercle

Enzymologic Data

As may be seen from an inspection of tables 1 and 2, the inflammatory zones surrounding seven-week-old tubercles showed markedly increased rates of enzymatic activity of LA and BAA. The values were approximately three times those for normal lung tissue. In areas which were not primarily involved, as judged by gross inspection, there was a moderate increase in hydrolysis. It is well to remember, however, that in these areas microscopic evidence of cellular infiltration was present, and that the tubercles at this time were not completely caseated but contained islands of intact inflammatory cells. Hence, these values are higher than those seen in normal lung tissue but lower than those in the surrounding zones of inflammation. While there was occasional microscopic evidence of softening in pulmonary tissue, there was an insufficient amount of softened material for chemical analysis seven weeks after infection.

TABLE 2

Hydrolysis of Benzoyl-l-Argininecamide and l-Leucinamide by Lung Homogenates of Normal and Tuberculous Rabbits

RABBIT NUMBER	LA-PEPTIDASE ACTIVITY			BA-AMIDASE ACTIVITY		
	$\frac{\text{mg. N}}{\text{ml. hom.}}$	K'_{LA}	C'_{LA}	$\frac{\text{mg. N}}{\text{ml. hom.}}$	$K_{BAA} \times 10^{-4}$	$C_{BAA} \times 10^{-4}$
Series A. Normal Rabbits						
6	0.532	0.89	1.7	3.14	4.4	1.4
6	0.588	0.86	1.5	1.56	1.4	0.9
6	0.375	0.69	1.8	1.50	2.7	1.8
96	1.900	2.30	1.2	1.90	3.0	1.7
1	0.518	0.72	1.4	2.68	3.0	1.1
Mean*		1.5 ± 0.11			1.4 ± 0.18	
Series B. Animals Killed 7 Weeks after Infection						
(a) Areas of Lung Not Primarily Involved						
492	0.299	0.58	1.9			
202	0.305	0.78	2.5	2.40	4.5	1.9
203	0.294	0.91	3.1	2.00	5.3	2.6
497	0.438	1.00	2.3	2.25	2.5	1.1
210	0.434	0.81	1.9	2.20	2.8	1.2
498	0.670	1.22	1.8	2.00	2.2	1.1
201	0.584	1.06	1.8	2.70	4.6	1.7
191	0.544	1.94	3.6	2.10	3.3	1.6
184	0.412	1.30	3.2	1.44	1.6	1.1
Mean		2.4 ± 0.23			1.5 ± 0.19	
(b) Inflammatory Zone Surrounding Tubercles						
202	0.151	0.88	5.8	1.69	8.3	4.9
492	0.290	1.42	4.9	2.47	13.0	5.2
201	0.224	1.20	5.4	2.22	9.4	4.2
497	0.216	0.86	4.0	2.00	5.4	2.7
210	0.310	1.10	3.6	1.80	4.4	2.5
203	0.280	2.30	8.2	2.10	13.0	6.2
203	0.121	1.50	12.0	1.80	18.4	10.0
192	0.170	1.00	6.0	2.18	12.0	5.5
199	0.211	1.40	6.8	2.68	9.7	3.6
189	0.255	1.70	6.7	1.89	8.0	4.0
Mean		5.8 ± 0.78			4.9 ± 0.67	
(c) Caseous Material in Center of Tubercles						
492	0.491	0.83	1.7			
202	0.600	1.37	2.2			
203	0.197	0.76	3.8			
492	0.438	0.89	2.0			
210	0.670	2.43	3.6	2.02	3.4	1.6
193	0.320	1.01	3.1	1.72	6.5	3.8
192	0.225	0.74	3.2	2.04	2.9	1.4
199	0.492	1.62	3.3	2.65	2.9	1.1
Mean		2.9 ± 0.30			2.0 ± 0.61	

TABLE 2—*Continued*

RABBIT NUMBER	LA-PEPTIDASE ACTIVITY			BA-AMIDASE ACTIVITY		
	$\frac{\text{mg. N}}{\text{ml. hom.}}$	K°_{LA}	C°_{LA}	$\frac{\text{mg. N}}{\text{ml. hom.}}$	$K_{BAA} \times 10^{-4}$	$C_{BAA} \times 10^{-4}$
Series C. Animals Killed 12 to 16 Weeks After Infection						
(a) Areas Not Primarily Involved						
489	0.530	1.24	2.30	2.75	4.1	1.6
488	0.590	1.10	1.90	2.79	4.0	1.4
498	0.510	1.12	2.20	2.23	4.6	2.1
489	0.516	1.22	2.30	2.05	5.3	2.6
207	0.558	1.63	2.90	2.67	3.0	1.1
207	0.441	1.20	2.70	2.70	6.1	2.2
211	0.568	1.85	3.30	2.50	4.4	1.7
209	0.425	1.42	3.34	2.06	4.8	2.3
212	0.530	1.80	3.40	2.88	11.6	4.0
490	0.372	0.90	1.63	2.55	4.3	1.7
Mean		2.56 ± 0.20			2.07 ± 0.2	
(b) Inflammatory Wall Surrounding Tubercles						
489	0.374	1.55	4.1	2.10	11.5	5.5
498	0.231	1.15	4.9	2.13	15.0	7.0
211	0.273	1.30	4.8	2.30	12.7	5.5
488	0.374	1.65	4.4	2.00	11.4	5.6
495	0.280	1.32	4.7	2.20	13.0	5.9
212	0.188	1.11	5.9	1.44	10.4	7.2
490	0.372	1.39	3.7	2.85	12.3	4.3
490	0.323	0.98	3.0	1.59	4.9	3.0
493	0.248	1.53	6.2	2.05	11.7	5.7
209	0.203	0.76	3.7	2.59	11.3	4.3
Mean		4.54 ± 0.33			5.4 ± 0.4	
(c) Caseous Material in Center of Tubercles						
211	1.75	1.54	0.87	4.27	1.1	0.20
491	1.60	0.56	0.35	3.14	1.8	0.57
491	0.82	0.49	0.60	2.72	0.3	0.10
208	1.40	0.29	0.20	2.65	4.7	1.80
209	1.51	0.92	0.61	3.44	1.2	0.30
209	1.20	0.41	0.34	2.87	1.9	0.70
Mean		0.49 ± 0.2			0.61 ± 0.11	
(d) Liquefied Material in Tubercles						
488	1.26	0.88	0.69	2.52	No hydrolysis	
488	0.88	0.69	0.78	1.76		
495	1.39	0.38	0.45	2.78		
495	1.40	0.89	0.63	2.80		
489	1.57	0.75	0.49	3.14		
212	0.99	0.55	0.55			
490	1.19	0.25	0.21			
Mean		0.54 ± 0.07				

TABLE 2—*Continued*

RABBIT NUMBER	LA-PEPTIDASE ACTIVITY			BA-AMIDASE ACTIVITY		
	$\frac{\text{mg. N}}{\text{ml. hom.}}$	K°_{LA}	C°_{LA}	$\frac{\text{mg. N}}{\text{ml. hom.}}$	$K_{BAA} \times 10^{-4}$	$C_{BAA} \times 10^{-4}$
Series D. Animals Killed 20 Weeks After Infection						
(a) Areas Not Primarily Involved						
205	0.430	1.02	2.1	2.30	4.1	1.8
205	0.630	1.43	2.3	2.90	5.4	1.9
204	0.620	1.55	2.5	2.90	6.3	2.1
206	0.420	1.05	2.5	2.00	3.0	1.5
496	0.510	1.09	2.2	2.30	3.1	1.4
Mean		2.3 ± 0.09			1.7 ± 0.13	
(b) Walls of Completely Softened Tubercles						
205	0.364	1.84	5.1	1.44	6.0	4.3
204	0.434	1.56	3.6	2.30	8.7	3.8
496	0.370	2.37	6.4	1.90	10.7	5.4
206	0.276	1.05	3.8	1.40	4.4	3.1
499	0.278	0.79	2.8	1.60	6.2	3.9
Mean		4.3 ± 0.6			4.1 ± 0.37	
(c) Liquefied Material in Tubercles						
205	1.54	0.39	0.25		No hydrolysis	
204	1.50	0.48	0.32			
204	1.36	0.25	0.18			
206	1.13	1.27	1.12			
206	1.04	1.13	1.08			
496	1.18	1.20	1.01			
496	1.71	0.38	0.22			
499	1.38	0.40	0.29			
Mean		0.56 ± 0.15				

Figures following ± are average deviation of the mean.
See footnotes to table 1.

Animals killed twelve to sixteen weeks after infection, in which caseation was much further advanced, showed little evidence of enzymatic hydrolysis of either substrate by homogenates of caseous material. The zones of inflammation surrounding these tubercles, as well as the areas which were not primarily involved, continued to show markedly increased rates of digestion. Nevertheless, liquefied material removed from softened tubercles had little hydrolytic activity for LA, and none whatever for BAA.

When the infection was allowed to progress for twenty weeks, hardly any normal lung tissue remained. The contents of liquefied tubercles had very little

capacity to hydrolyze LA and there was complete absence of activity for BAA. Enzymatic hydrolysis by the "wall" of completely softened tubercles and by tissue removed from areas not primarily involved remained essentially as active as in animals killed at earlier intervals.

Activators of Endocellular Proteinases

A review of the foregoing data (tables 1 and 2) reveals that, while there is evidence of greatly increased BA-amidase and LA-peptidase activity in the inflammatory zones surrounding tubercles, nevertheless, whenever there is gross evidence of caseation in the centers of these tubercles, the rate of substrate cleavage is markedly decreased. The values for BA-amidase reach zero in areas where microscopic softening has developed. In sections of lung intermediate

TABLE 3

*Results of Qualitative Tests for the Presence of Sulfhydryl Groups**

	TEST FOR FREE CYSTEINE			TEST FOR GLUTATHIONE	
	$FeCl_3$	$CuSO_4$	Sodium nitroprusside	1, 2, naphthoquinone	Sodium nitroprusside
Homogenate of normal lungs	−	−	−	−	+
Homogenate of tuberculous lungs not primarily involved	−	−	−	−	+
Homogenate of inflammatory walls of tubercles	−	−	−	−	+
Liquefied material from softened tubercles	−	−	−	−	−
Caseous material from tubercles	−	−	−	−	−

* Technique used in these tests is that given by Hawk, Oser, and Summerson (25, pp. 129, 145, and 283).

between primary tubercles, where there are only microscopic areas of cellular infiltration, the increase in the rate of enzymatic hydrolysis is only moderate. It appears, therefore, that the rate of proteinase digestion is proportional to the intensity of cellular infiltration. This observation is an extension of previous work (9, 10) in which it was shown that total organ homogenates (lungs and kidneys) of rabbits, which had been primarily infected with virulent tubercle bacilli and in which there is an intense inflammatory reaction, possess a more rapid rate of enzymatic hydrolysis of BAA than those from normal organs. The data further suggest that, wherever there are intact cells, there is proteinase action; and, on the other hand, wherever the cells have undergone necrobiosis (caseation or softening), there is decrease or absence of enzymatic hydrolysis.

The question naturally arises: Is it possible that the decrease in the capacity of caseous and liquefied material to hydrolyze BAA and LA is due not only to loss of enzymes but also to the absence of activators or the presence of inhibitors, or the operation of both factors? Various tests (table 3) have shown that both caseous tissue and material removed from softened tubercles are devoid

of free sulfhydryl groups and hence of free cysteine and glutathione which function as activators. Nevertheless, when an excess of cysteine or of Mn^{++} ions was supplied as activator, no augmentation in the rate of hydrolysis was obtained with either BA-amidase or LA-peptidase.

DISCUSSION

In the present investigation it has been possible to demonstrate a complete absence of BA-amidase and a marked decrease in LA-peptidase activity in softened tubercles of the lungs of rabbits. A marked decrease in the rate of hydrolysis of both substrates was also observed in the caseous centers of discrete tubercles and an intense augmentation of enzymatic hydrolysis in the inflammatory zones surrounding them.

Whether or not these findings are unique to the tuberculous process or are present in inflammatory tissue produced by other infections or irritants is unknown and should be a subject for future investigation. In this connection, it is of interest to note that similar enzymatic changes have been reported in a variety of pathologic processes which are accompanied by injury to, or destruction of, cells. Thus Purr (18), confirming the work of Waldschmidt-Leitz and McDonald (19) and Maschmann and Helmert (20), showed that cathepsin is practically absent in the necrotic portions of animal tumors. Likewise, when kidneys are severely injured by ligation of their arterial circulation, there is a marked reduction in their capacity for catheptic hydrolysis (21). Finally, when animals are exposed to untoward influences such as phosphorus or carbon tetrachloride poisoning (22) or starvation (23), there is a marked decrease or loss of endocellular proteinases from the damaged organs. As shown above in the case of liquefied tubercles, so also in the necrotic areas of animal tumors the sulfhydryl concentration drops nearly to zero (24).

SUMMARY

Tubercles were produced in the lungs of unselected albino rabbits by means of the Wells apparatus for airborne droplet nuclei infection which permits the simultaneous exposure of animals in groups to predetermined doses of virulent tubercle bacilli. The animals were killed at varying intervals of time after infection, thus permitting observations during successive stages in the development of discrete tubercles.

Employing recently developed enzymologic procedures, examinations were made of the rates of hydrolysis of benzoyl-l-arginine amidase and leucine-amino peptidase by homogenates of material removed from the interior of liquefied tubercles, from the centers of caseation, and from the zones of inflammation which surround tubercles. These data on proteinase activity were correlated with the histologic findings on comparable specimens.

There was evidence of greatly increased enzymatic activity (BA-amidase and LA-paptidase) in the inflammatory zones of the tubercles. The areas of lung

intermediate between primary tubercles which contained only microscopic areas of cellular infiltration showed only a moderate increase in the rate of enzymatic hydrolysis. In the centers of the tubercles where there was gross evidence of caseation, the rate of subetrate cleavage was markedly decreased. The values for the rate of hydrolysis of LA became very low and those for BAA reached zero in areas where macroscopic softening had developed.

It appears, therefore, that the rate of proteinase action is proportional to the intensity of cellular infiltration. The data further suggest that wherever there are intact cells there is proteinase action and, on the other hand, wherever the cells have undergone necrobiosis (caseation or softening) there is a decrease or absence of enzymatic hydrolysis.

It is tentatively suggested that decreases in proteinase activity may be due, not only to the loss of certain enzymes and their activators, but also to the presence of inhibitors which inactivate or denature these enzymes (26).

SUMARIO

Sobre el Mechanismo de Licuación de los Tubérculos: El Comportamiento de las Proteinasas Endocelulares en los Tubérculos Formados en los Pulmones de los Conejos

Empleando procedimiento enzimológicos recién elaborados, hiciéronse estudios de la velocidad de la hidrólisis de BAA y LA por homogenatos de substancias extraídas del interior de tubérculos licuados, de los centros de caseación y de las zonas de inflamación que rodean a los tubérculos. Estos datos relativos a la actividad de las proteinasas fueron correlacionados con los hallazgos histológicos en ejemplares homólogos.

Hubo signos de considerable aumento de la actividad enzimática (BA-amidasa y LA-peptidasa) en las zonas inflamatorias de los tubérculos. Las zonas pulmonares intermedias entre los tubérculos primarios que sólo contenían zonas microscópicas de inflamación celular no mostraron más que aumento moderado de la velocidad de la hidrólisis enzimática. En los centros de los tubérculos en que había signos macroscópicos de caseación, la velocidad de la desintegración subestratal había disminuído notablemente. Las cifras de la velocidad de la hidrólisis de LA eran muy bajas y las de BAA llegaban a cero en las zonas en las que había reblandecimiento macroscópico.

Parece, pues, que la intensidad de la acción proteinásica se halla en proporción a la de la infiltración celular. Estos datos indican que, dondequiera que haya células intactas, hay acción proteinásica, y, por otra parte, siempre que las células hayan experimentado necrobiosis (caseación o reblandecimiento), disminuye o falta hidrólisis enzimática.

Indícase tentativamente que las disminuciones de la actividad de las proteinasas pueden deberse no sólo a la pérdida de ciertos enzimas y de los activadores de los mismos, sino también a la presencia de inhibidores que inactivan o desnaturalizan dichos enzimas.

RESUME

A propos du mécanisme de la liquéfaction des tubercules: comportement des protéinases endocellulaires dans le tubercle se développant dans le poumon du lapin

A l'aide de procédés enzymologiques récemment mis au point, il a été procédé à des déterminations du taux d'hydrolyse de BAA et de LA en utilisant des substances homogénéisées provenant de tubercules liquéfiés et prélevés au niveau du centre de caséification et de la zone inflammatoire qui entoure les tubercules. Ces données sur l'activité des protéinases correspondaient aux résultats de l'examen histologique des spécimens homologues.

Des signes d'une activité enzymatique considérablement accrue (BA-amidase et LA-peptidase) ont été trouvés dans la zone inflammatoire des tubercules. Au niveau des régions du poumon situées entre les tubercules primaires et ne contenant que de microscopiques parties d'infiltration cellulaire le taux d'hydrolyse enzymatique ne subissait qu'une augmentation modérée. Au centre des tubercules où la caséification était très marquée, le taux de clivage du substrat était nettement diminué. Les valeurs relatives au taux d'hydrolyse de LA se sont beaucoup abaissées et celles pour le BAA sont tombées à zéro dans les zones où existait un ramollissement macroscopique.

Par conséquent, il semble que le taux de l'action de la protéinase soit proportionnel à l'intensité de l'infiltration cellulaire. Ces données suggèrent qu'une action de la protéinase se fait sentir en tout endroit où il y a des cellules intactes et que, d'autre part, partout où les cellules se nécrosent (caséification et ramollissement) l'hydrolyse enzymatique décroît ou est absente.

On suppose que cette diminution de l'activité des protéinases peut-être due, non seulement à la perte de certaines enzymes et de leurs activateurs, mais aussi à la présence d'un ou de plusieurs inhibiteurs qui inactivent ou dénaturent ces enzymes.

Acknowledgments

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Case Reports

BILATERAL TUBERCULOUS BRONCHOSTENOSIS IN A PATIENT WITH NORMAL CHEST ROENTGENOGRAPHIC FINDINGS[1, 2]

JOHN S. PACKARD

(Received for publication March 1, 1951)

Tuberculous involvement of the major bronchi has been widely recognized in recent years, and treatment of this complication of pulmonary tuberculosis has been greatly aided by streptomycin and other antituberculous drugs. Unfortunately, stenosis of one of the larger bronchi from endobronchial tuberculosis still occurs rather frequently and often leads to the need for excisional pulmonary surgery.

The following case report is concerned with the first bilateral bronchostenosis observed by the writer. Of additional interest is the fact that the patient has had normal chest roentgenographic findings during the entire period of observation. While unilateral bronchostenosis has frequently been reported, bilateral stenosis is apparently rather rare.

I. C., a 65-year-old white housewife, native of Pennsylvania, was in good health until March 1949 when she had an attack of bronchitis and laryngitis. Cough and hoarseness continued and her physician ordered a chest roentgenogram in April which was reported as being normal. In July the patient was referred for bronchoscopy. Bleeding from the right main bronchus was reported during the examination, but no localized lesion could be seen. Bronchial secretions were found to contain acid-fast bacilli and the patient was referred to the sanatorium.

The patient was admitted to this service on August 14, 1949. She still complained of cough and bronchial irritation. The sputum was positive for acid-fast bacilli (Gaffky VI) on direct smear. Culture of the sputum resulted in a growth of colonies characteristic of the human type of *M. tuberculosis*. Material from this culture was inoculated into guinea pigs by the Department of Bacteriology, Bucknell University. Typical involvement of the lymph nodes and spleens of these animals was demonstrated and acid-fast bacilli were recovered from these organs.

Stereoscopic chest films on admission (figure 1) were normal in appearance except for small calcified nodules in the left hilar region. Lateral films and planigrams revealed no abnormal findings at any level, behind the heart or behind the domes of the diaphragm.

Serial films taken at monthly intervals until August 1950 showed no abnormalities.

A six-week course of dihydrostreptomycin in daily doses of one gram was given, followed by sodium para-aminosalicylate which was continued to August 1950. Two more six-week courses of dihydrostreptomycin combined with the sodium PAS were given at intervals.

Bronchoscopic examination: Bronchoscopy, August 31, 1949, showed diffuse redness and swelling of the mucosa of both main bronchi, and the slightest touch with the aspirator resulted in considerable bleeding which prevented further examination.

A second bronchoscopy performed on January 18, 1950 showed that the mucosa of both main bronchi was much smoother and paler in appearance and only the slightest amount of bleeding was noted. Moderate narrowing of both main bronchi was present, being more marked just below the upper orifices.

A third bronchoscopy on May 17, 1950 showed the bronchial mucosa to be only slightly

[1] From Devitt's Camp, Allenwood, Pennsylvania.

[2] A preliminary report on this case was given before the Pennsylvania Trudeau Society in May 1950.

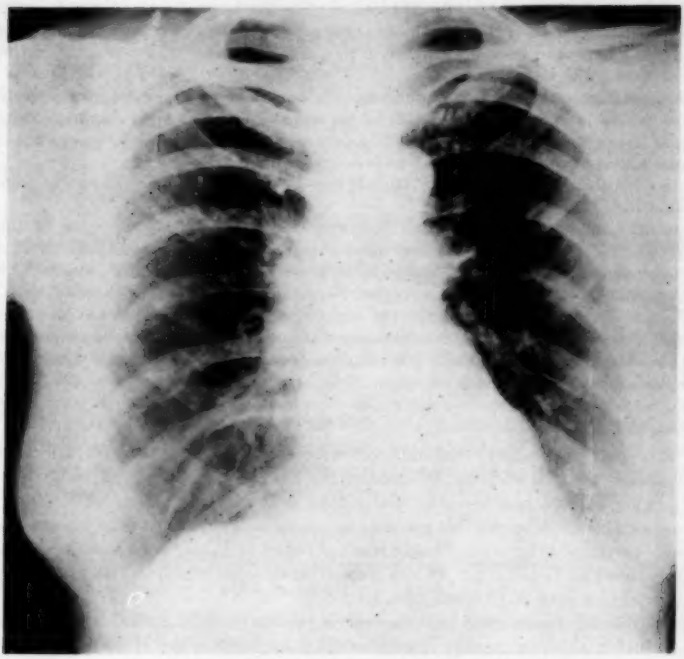

FIG. 1. Roentgenogram taken on admission in August 1949

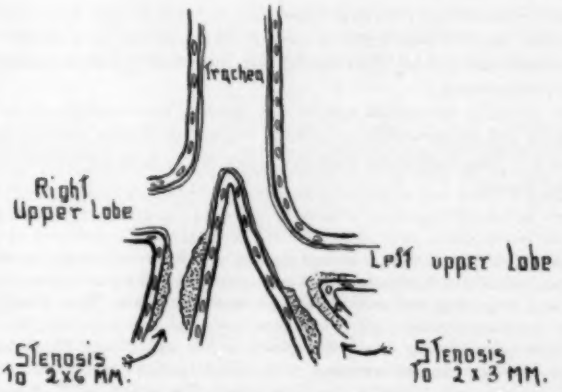

FIG. 2. Showing bilateral bronchostenosis, sketched from bronchoscopic findings

reddened, and there was virtually no bleeding. The left upper lobe orifice was patent but somewhat narrowed, and its spur thickened. Just below the upper lobe the left bronchus was greatly narrowed to approximately 2 by 3 mm., and the aspirator could not be advanced through the stenosis.

The right main bronchus was considerably narrowed at a point approximately one centimeter below the right upper lobe bronchus. At this point the bronchial lumen was reduced to approximately 2 by 6 mm. The aspirator was easily passed through the stenosis, and no retained secretions were encountered (figure 2).

A fourth bronchoscopy on August 16, 1950 showed no essential change from the preceding examination.

Sputum examinations at monthly intervals showed a gradual reduction in tubercle bacilli, and all examinations of direct smears were negative after June 1950. Culture of a three-day concentrate in July 1950 showed no growth for tubercle bacilli. Bronchial secretions obtained at bronchoscopy on August 16 were also negative for tubercle bacilli.

The patient was discharged from the sanatorium in apparent good health on August 20, 1950, with recommendations for continued rest and serial follow-up by a chest specialist. In spite of her rather marked bilateral bronchostenosis she had little or no dyspnea at rest or on walking slowly on the level.

COMMENT

The ultimate prognosis of bilateral bronchostenosis in tuberculosis is certainly in doubt. It is possible that the early use of antimicrobial agents may have considerably improved the prognosis in the case reported. Early treatment may have postponed or prevented the appearance of demonstrable parenchymal involvement and extreme degrees of stenosis with resulting suffocation. Plastic surgical repair of tuberculous bronchostenosis has been reported by Gebauer (1, 2), and further work along this line may make such procedures feasible even in bilateral cases.

Certainly such repair could be considered in persons exhibiting little or no parenchymal disease and in whom pulmonary resection need not be carried out. If the degree of stenosis in the present case becomes more severe, plastic surgery could be life saving.

SUMMARY

The case of a patient with bilateral tuberculous bronchostenosis has been reported. No previous similar case has been found in medical literature (see addendum).

In the present case normal chest roentgenographic findings were present throughout the period of observation.

The early use of antimicrobial agents may prevent the development of increasingly severe stenosis and of parenchymal spreads. Moreover, plastic surgical repair of progressively severe bronchostenosis may be considered in cases exhibiting inactive or no parenchymal disease.

Addendum

Since this paper was written, a second patient with bilateral bronchostenosis has been seen. A young woman was admitted in September 1950, with tuberculous endobronchial ulceration and beginning stenosis of the left main bronchus. This young woman had had a right pneumonectomy eight years previously for complete stenosis of the right main bronchus complicating total tuberculosis of the right lung. The bronchial lesions have shown considerable improvement with antimicrobial therapy and no increase of stenosis in the left main bronchus has been noted. The severe initial dyspnea has been somewhat reduced. Surgical repair in this case would present very great technical difficulties.

SUMARIO

Broncoestenosis Tuberculosa Bilateral en un Enfermo con Hallazgos Radiográficos Normales en el Tórax

Comunícase un caso de broncoestenosis bilateral. No se ha encontrado ningún caso semejante en la literatura médica (addendum).

El caso actual reveló hallazgos radiográficos normales en el tórax durante todo el período de observación.

El empleo temprano de agentes antibióticos y quimioterapéuticos puede impedir la aparición de estenosis cada vez más intensa y de difusión al parénquima.

En los casos que no manifiestan enfermedad parenquimática o la muestran inactiva, cabe considerar la cirugía plástica para la broncoestenosis en vías de agravación.

Addendum

Ya escrito este trabajo, ha sido observado otro enfermo con broncoestenosis bilateral. Una joven fué recibida en el mes de septiembre de 1950 con ulceración endobronquial tuberculosa y estenosis incipiente del principal bronquio izquierdo. Esta joven había experimentado una neumonectomía derecha ocho años antes por estenosis total del bronquio mayor derecho que complicó una tuberculosis total del pulmón drecho. Con la antibioticoterapia se ha observado considerable mejoría sin aumento de la estrechez del principal bronquio izquierdo. También se ha atenuado algo la intensa disnea inicial. En este caso, la cirugía plástica entrañaría dificultades técnicas muy grandes.

RESUME

Sténose bronchique tuberculeuse bilatérale chez un malade avec image radiologique pulmonaire normale sans signe radiologique d'atteinte pulmonaire

Un cas de sténose bronchique bilatérale a été rapporté. Aucun cas semblable n'a été trouvé dans les publications médicales (addendum).

Les images radiologiques du cas présenté ont été normales pendant toute la durée de l'observation.

L'usage précoce d'agents antibiotiques et chimiothérapiques peut prévenir le développement d'un processus sténosant grave et l'extension des lésions au parenchyme.

Une intervention plastique pour pallier une sténose bronchique marquée peut être envisagée dans les cas stabilisés ou sans atteinte du parenchyme pulmonaire.

Addendum

Depuis la publication de cette communication un second malade atteint de sténose bronchique bilatérale a été vu. Il s'agit d'une jeune femme admise en September 1950, elle présentait une ulcération tuberculeuse endobronchique avec début de sténose au niveau de la bronche souche gauche. Cette jeune femme avait subi huit mois auparavant une pneumectomie droite pour oblitération complète de la bronche souche droite compliquant une tuberculose envahissant la totalité du poumon droit. Une amélioration considérable a été observée à la suite du traitement antibiotique et la sténose de la bronche souche gauche n'a pas augmenté. La dyspnée sévère initiale a un peu diminué. Dans ce cas une intervention plastique présenterait de grandes difficultés techniques.

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PNEUMOTHORAX AS A COMPLICATION OF PNEUMOPERITONEUM[1]

JACK FOY WU AND WILFORD B. NEPTUNE

(Received for publication February 14, 1951)

The use of therapeutic pneumoperitoneum in the management of pulmonary tuberculosis is generally thought to be a relatively benign procedure. Although the complications which theoretically might occur are numerous, their actual occurrence is not too common.

The following case is presented to demonstrate an unusual complication of therapeutic pneumoperitoneum.

CASE REPORT

E. D. (Case #5176), a 40-year-old white female, was referred to the American Legion Hospital on September 1, 1950 following severe hemoptysis.

After medical observation for five months, a diagnosis of pulmonary tuberculosis had been made in September 1949 and she entered a hospital, but left against advice after seven months of bed rest. During a follow-up examination she had an attack of hemoptysis, and was referred to this hospital.

At the time of admission she admitted the presence of cough, production of a small amount of sputum, and weakness, in addition to the recent hemoptysis. Moreover, she related that a diagnosis of diabetes mellitus had been made during the previous hospitalization. Treatment, with apparent control at that time, had consisted of diet regulation only.

Physical examination revealed a well-developed, rather obese, white female. Temperature was 99°F., respirations 20, and pulse 90 per minute. The blood pressure was 110 systolic, and 70 diastolic in mm. of mercury. The only other pertinent finding was the presence of moderately coarse rales heard throughout the left hemithorax.

Laboratory examinations revealed a total erythrocyte count of 4,350,000 per cu. mm., and 12.95 gm. of hemoglobin per 100 cc. The total leukocyte count was 11,000 per cu. mm., with a normal differential count. The erythrocyte sedimentation rate was 0.2 mm. per minute (Rourke and Ernstene). The urine had a specific gravity of 1.020; contained no albumin; yielded a 4 plus reaction for sugar, and was negative for acetone. Microscopic examination of the sediment was negative. A serologic test for syphilis was negative. The blood sugar was 218 mg. per 100 cc. The blood type was O, Rh negative. The sputum examinations by direct smear were negative for acid-fast bacilli; however, cultures were positive for *M. tuberculosis*.

Roentgenographic examination of the chest disclosed a cervical rib on the left side. In the left hemithorax, the upper one-third was involved with an area of increased density, which apparently represented a mixed type of infiltration. Beneath the clavicle there was an elliptical area of decreased density which was thought to represent cavity.

Following admission to the hospital, the patient was placed on para-aminosalicylic acid, 12 gm. per day; and dihydrostreptomycin every third day (in a daily dosage of 1.0 gm.) for a total period of 120 days. Her diet consisted of 900 gm. of carbohydrate, 100 gm. of protein, and 100 gm. of fat. Soon after admission the blood sugar rose to 361 mg. per 100 cc., and she developed acetonuria. She was then given insulin and, following regulation, 70 units of 2:1 mixture of regular and protamine zinc insulin were required for maintenance. On this regimen the urine was negative for sugar and acetone, and the blood sugar varied between 117 and 147 mg. per 100 cc. A second roentgenographic examination of the chest on October 14, 1950, following six weeks of bed rest, chemotherapy, and regulation of the

[1] From the American Legion Hospital, Battle Creek, Michigan.

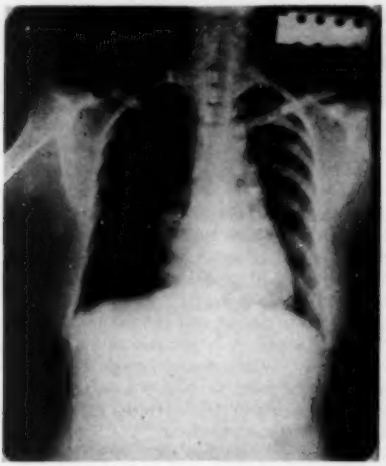

FIG. 1. Roentgenogram of the chest, following two pneumoperitoneum treatments, showing a right pneumothorax and complete absence of intraperitoneal air.

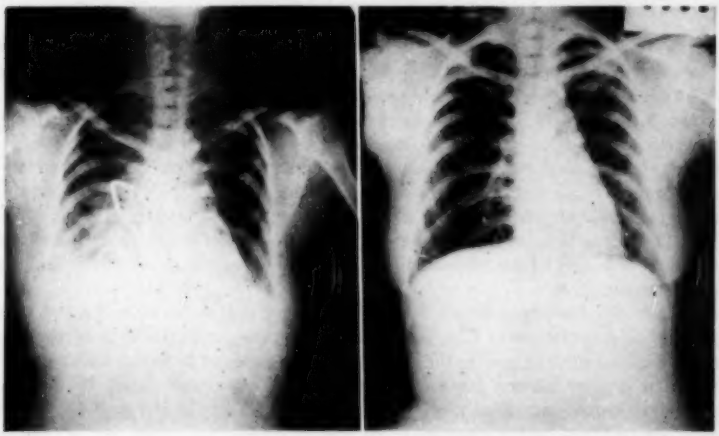

FIG. 2. (Left) Roentgenogram of the chest, four hours following decompression of the right spontaneous pneumothorax.

FIG. 3. (Right) Roentgenogram of the chest, two days following the spontaneous pneumothorax with re-expansion of the right lung and complete absence of intraperitoneal air.

diabetes, showed definite clearing of the parenchymal lesions. At this time therapeutic pneumoperitoneum was recommended.

Induction of pneumoperitoneum: Pneumoperitoneum was induced on October 23, 1950, 750 cc. of air were given through a left para-umbilical location, and the closing pressures

were plus 8, plus 10. Postpneumoperitoneum fluoroscopy revealed air beneath the domes of the diaphragm. The second treatment was given on October 26, 1950, and consisted of 1,000 cc. of air; opening pressure readings were not obtained, and on closing they were plus 10, plus 12. Pre- and post-pneumoperitoneum fluoroscopy again showed only the presence of air beneath the domes of the diaphragm with no unusual findings.

A short time following the refill (three days following initial treatment), the patient developed some dyspnea, but this was not severe and was attributed to anxiety. On October 28, 1950, she developed severe dyspnea with the absence of breath sounds over the right hemithorax. Fluoroscopy revealed no evidence of pneumoperitoneum, but a right pneumothorax with almost complete collapse of the lung. Roentgenographic examination (figure 1) revealed confirmation of these findings. There was no evidence of mediastinal emphysema and no evidence of a left pneumothorax.

It was the clinical impression that the patient obviously had a communication between the peritoneal and right pleural cavities. In view of the dyspnea, collapse of the good lung, and her diabetes, it was decided to decompress the pneumothorax as fast as possible; this was accomplished by the insertion of an intercostal catheter in the anterior second right intercostal space and attached to water-seal drainage. Decompression was followed by immediate relief. Four hours later the fluctuations ceased in the water-seal tube, and roentgenographic examination (figure 2) demonstrated complete re-expansion of the right lung. The intercostal catheter was then removed.

The clinical condition progressed satisfactorily, with the exception of a mild upset in the control of her diabetes which again required regulation.

Roentgenographic examination of the chest (figure 3) on October 30, 1950 revealed complete expansion of the right lung with no evidence of pneumoperitoneum. Follow-up examination during the next three months revealed no additional complications.

COMMENT

The criterion for the diagnosis of spontaneous pneumothorax complicating pneumoperitoneum is an associated loss of intraperitoneal air. In all of the twenty cases previously reported (1, 2, 3, 4, 5, 6), the pneumothorax always occurred on the right side. As pointed out by Ross and Farber (6), this would seem to be associated with the displacement of the liver incident to the use of pneumoperitoneum. Other precipitating factors may be indirect trauma or marked increase in intra-abdominal pressure. The most likely anatomic basis for passage of air into the thorax is thought to be through a congenital diaphragmatic defect. These defects occur due to failure of proper fusion of the component parts of the diaphragm, or to lack of the mesoderm being properly deposited at any of these adjacent points of union (7, 8).

Obviously the size of the defect determines to some degree the time of onset for the passage of air into the thorax. The larger the opening, the earlier it will be apparent; the smaller, the later and the greater the likelihood of being related to factors causing marked increase in intra-abdominal pressure. Following diagnosis there should be immediate symptomatic decompression as needed. In the final disposition of the problem, there are several alternatives: discontinuance of the pneumoperitoneum, which is the procedure of choice in those cases occurring early and due most likely to a large defect; reinstitution of the pneumoperitoneum after complete re-expansion of the lung, a course which may be pursued when it is believed that the defect is small, is related to the increased intra-abdominal pressure, and that pleural symphysis will occur, thereby further aiding in preventing recurrence of the spontaneous pneumothorax; and, finally, conversion to therapeutic pneumothorax if the parenchymal lesion warrants such therapy.

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Notes

THE VIABILITY OF BCG VACCINE

One of the most serious technical difficulties in the use of BCG vaccines lies in their very short effective life. Most authorities recommend that liquid vaccines should be used within ten days of preparation, and figures such as those recorded by Aronson and Schneider (Am. J. Pub. Health, 1950, *40*, 533) suggest that even ten days may be too long a period. Freeze-dried vaccines are better but, where reasonably accurate viable counts have been made before and after drying (Birkhaug, Am. J. Pub. Health, 1950, *40*, 545; Böe and Greaves, Acta tuberc. Scandinav., 1950, *24*, 38), a considerable immediate loss in viable organisms (of the order of 90–99 per cent) has been reported.

Last year a report was published of the results obtained when BCG was grown diffusely in a liquid medium containing Tween 80 and a filtrate of heated human serum (Dubos and Fenner, J. Exper. Med. 1950, *91*, 261; Fenner and Dubos, J. Exper. Med., 1950, *91*, 269). The viable count of the liquid vaccine after six weeks' storage in the refrigerator was as high as that of the fresh preparation, and the potency in guinea pigs of the fresh and stored vaccines was identical.

Since that time further investigations have been made of the longevity of BCG grown and stored in various ways. Counts of the number of viable bacterial units in liquid and freeze-dried preparations were made after various intervals of storage in the refrigerator and at room temperature, which was regularly recorded and varied from 15° to 30°C. (usually 20° to 25°C.) during the period. The counts were made on the surface of oleic-albumin-agar (Fenner, Martin, and Pierce, Ann. New York Acad. Sc., 1949, *52*, 751), at least ten replicates being made at each of the appropriate dilutions. On every occasion on which plate counts were made, stained smears of the preparation were examined and differential counts made to determine the state of dispersion of the bacteria. This was always reasonably good (approximately 60 per cent single bacilli and 8 per cent clumps of more than 5 bacteria), and for individual preparations it remained remarkably constant throughout the experiments. A summary of the viable counts obtained is recorded in the table.

The outstanding feature was the slight fall in the viable counts found with cultures grown in Tween-albumin medium, whether stored at refrigerator or room temperature. A limited analysis of the factors responsible for the rapid dying off of bacilli in standard vaccines, compared with their prolonged viability in the Tween-albumin medium, indicates that a small proportion of the bacilli which die in the standard vaccine can be protected for months by the inclusion of 0.5 per cent bovine albumin in the suspending medium (experiment A). That lack of this protective agent is probably not the major factor in the dying off is suggested by experiment C, in which it was found that bacilli, grown in Tween-albumin medium, washed, and resuspended in Sauton's medium diluted to one-quarter strength, survived almost unchanged two weeks later, at a time when the count of the pellicle growth suspended in Sauton had fallen by 2 $\log_{10}$ units.

When large inocula were employed, good dispersed growth of BCG was obtained in Dubos liquid medium which lacked albumin (experiment D), and the viable count of such a culture was maintained much better than is the case with the standard vaccines. This vaccine, obtained from the albumin-free Dubos medium, produced tuberculin allergy in guinea pigs (positive to 1:1,000 Old Tuberculin three weeks after vaccination) after a month's storage at room temperature.

Viable Counts on Various Preparations of BCG Vaccine

EXPERIMENT	CULTURE MEDIUM	STORED AS	VIABLE COUNTS (COLONIES/ML) AT VARIOUS INTERVALS								TEMPERATURE OF STORAGE
			Original	Immediately after freeze-drying	Weeks				Months		
					1	2	3	4	2	3	4	
A	Sauton (pellicle)	Liquid vaccine in ¼ Sauton	$10^{7.0}$		$10^{6.3}$	$10^{4.7}$	$10^{4.7}$	$10^{3.1}$	$10^{1.3}$	0		4°C
					$10^{6.1}$	$10^{5.2}$	$10^{4.7}$	$10^{2.0}$	0			R.T.*
	Sauton (pellicle)	Liquid vaccine in 0.5 per cent bovine albumin in distilled water	$10^{5.9}$		$10^{5.8}$	$10^{4.6}$	$10^{4.2}$	$10^{3.7}$	$10^{3.7}$	$10^{2.2}$		4°C
					$10^{4.7}$	$10^{3.7}$	$10^{2.6}$	$10^{2.0}$	$10^{2.7}$	0		R.T.
B	Tween 80—bovine albumin—basal	Liquid vaccine in original culture medium	$10^{8.4}$		$10^{8.5}$	$10^{8.2}$	$10^{8.1}$	$10^{8.1}$	$10^{7.9}$	$10^{6.5}$		4°C
					$10^{8.5}$	$10^{8.1}$	$10^{8.0}$	$10^{7.9}$	Contaminated			R.T.
	Tween 80—bovine albumin—basal	Spun down and resuspended in 0.5 per cent bovine albumin in distilled water	$10^{8.4}$		$10^{8.0}$	$10^{8.0}$	$10^{7.8}$	$10^{7.8}$	$10^{7.3}$	$10^{7.0}$		4°C
					$10^{8.1}$	$10^{7.9}$	$10^{7.4}$	$10^{7.3}$	$10^{6.6}$	$10^{6.3}$		R.T.
C	Tween 80—bovine albumin—basal	Liquid vaccine in original culture medium	$10^{8.2}$			$10^{8.3}$	$10^{6.9}$					4°C
	Tween 80—bovine albumin—basal	Spun down and resuspended in ¼ Sauton	$10^{8.0}$		$10^{8.0}$	$10^{8.2}$	$10^{6.7}$					4°C
D	Tween 80—basal	Liquid vaccine in original culture medium (no albumin)	$10^{8.0}$					$10^{6.9}$				R.T.
E	Tween 80—bovine albumin—basal	Liquid vaccine in original culture medium	$10^{8.4}$					$10^{7.7}$	$10^{8.4}$	$10^{7.4}$	$10^{7.1}$	4°C
								$10^{7.8}$	$10^{8.0}$	$10^{7.2}$	$10^{6.7}$	R.T.
	Tween 80—bovine albumin—basal	Freeze-dried from original culture medium	$10^{8.4}$	$10^{7.8}$				$10^{7.6}$	$10^{7.4}$	$10^{7.4}$	$10^{7.4}$	4°C
								$10^{7.1}$	$10^{7.1}$	$10^{7.1}$	$10^{7.1}$	R.T.
	Tween 80—bovine albumin—basal	Freeze-dried from original culture medium containing in addition 50 per cent glucose	$10^{8.0}$	$10^{7.5}$				$10^{7.0}$	$10^{7.4}$	$10^{6.2}$	$10^{6.0}$	4°C
								$10^{6.1}$	$<10^{2}$	0	0	R.T.

* R.T. = room temperature (15°-30°C., usually 20°-25°C.)

As Dr. E. A. North pointed out to the writer, cultures grown in the Tween-albumin medium freeze-dry very well, and resuspend in an instant upon the addition of distilled water to give a very well dispersed vaccine. Viable counts indicated that the addition of glucose to the medium to give a final concentration of 50 per cent glucose produced no improvement in either the immediate loss on freeze-drying (71 per cent without glucose, 70 per cent with glucose) or the maintenance of the viable count, especially in the preparations kept at room temperature. In addition, resuspension of the glucose preparation is quite laborious, for it takes at least ten minutes to resuspend satisfactorily the contents of an ampoule of dry glucose vaccine. It will be noted, however, that the viable counts of the liquid cultures are maintained at a somewhat higher level than the dried material, even when stored at room temperatures, until after the fourth month. The effect on the liquid and dried vaccines of prolonged exposure to temperatures of 37°C. and more was not studied.

Vaccination of guinea pigs with the preparations used in experiment E, four months after preparation of the cultures, showed that all produced tuberculin allergy (positive reaction to 1:1,000 OT three weeks after vaccination) except the dried glucose vaccine which had been stored at room temperature.

A more detailed analysis of the factors responsible for the rapid destruction of bacilli in standard vaccines is in preparation. The present preliminary communication, which suggests that factors involved in pellicle growth and the grinding of the bacteria in a ball mill are perhaps the most important, is made so that those responsible for BCG production may be encouraged to take advantage of the highly potent and durable product obtained by dispersed growth of BCG in Dubos liquid medium.

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February 28, 1951

IMMERSION OIL AS A POSSIBLE SOURCE OF DIAGNOSTIC ERRORS

The procedure of examining slides microscopically for tubercle bacilli contains many pitfalls. Among these are the presence of saprophytic acid-fast bacilli in the water used, and the presence of acid-fast bacilli on previously used and improperly cleaned slides or on the surface of the microscope lens. Artifacts, such as scratches or imperfections in the slides, or paraffin particles in the specimen, which may retain the fuchsin stain after decolorization, may simulate acid-fast bacilli.

Recently another unsuspected source of extraneous contaminating acid-fast bacilli has been found. In the course of examining a slide stained with Löffler's methylene blue, occasional red-staining rods having the morphology of the tubercle bacillus were noted. Since this slide had not been used previously and since no fuchsin or pyronin had been used in the staining process, a search was made for the source of contamination. It was found that the applicator used in applying immersion oil had been contaminated by contact with slides carrying smears of acid-fast bacilli, and that adhering acid-fast bacilli had been transferred by the applicator to other slides and to the oil.

Attention is called to the obvious consequences of such contamination from a diagnostic standpoint. It is strongly recommended that immersion oil be applied only by some disposable type of applicator such as a toothpick, which may be used once and then discarded.

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April 11, 1951

AN IMPROVED ULTRAFILTRATION APPARATUS[1, 2, 3]

Nitrocellulose membranes have long been used for concentrating aqueous solutions of proteins, and the most generally used procedure is to form the membrane on an alundum extraction thimble. A procedure for using the filters has been described by Seibert (Am. Rev. Tuberc., 1934, *30*, 713) and Kabat and Mayer (Experimental Immunochemistry, Charles C Thomas, Springfield, Ill., 1948), but the equipment is cumbersome. A large amount of bench space is required because great care must be taken not to scratch the surface of the filter.

The necessity of concentrating large volumes of dilute protein solutions resulted in the development in this laboratory of an apparatus which would handle several liters per day and yet would require a minimum of laboratory space. The modification also facilitated reduction of the final concentrate to a small volume.

The most important feature of the apparatus is the introduction of a glass mantle to surround the filter proper. Figure 1 is a photograph of the apparatus in operation, and figure 2 shows more clearly the details of the construction and assembly of the filter unit. The exact location of the two outlets is not critical, and the clearance between the filter membrane and the inside of the glass mantle need be only a few millimeters.

Two sizes of alundum thimbles were used, depending on the amount of fluid to be handled. The mantles for the larger-sized thimbles, 45 by 127 mm., were constructed from 57 mm. pyrex tubing, and for the small size, 23 by 80 mm., from 30 mm. tubing. The final volume of concentrate in the filter assemblies is approximately 50 ml. and 15 ml., respectively. Extraction thimbles of medium porosity, RA 360, have been found to be satisfactory, but both coarser and finer thimbles can be used. Kabat and Mayer (*supra*) recommend a specific pyroxylin but other brands or types of nitrocellulose can be used. It is true that the type of nitrocellulose used in preparing the membrane is very important and may account for the wide variation in results obtained by different investigators. It has been reported by Asheshov (J. Bact., 1933, *25*, 339) that the permeability varied as much as 25 per cent when different lots of the same brand of nitrocellulose were used. Consequently, it is important that each lot be carefully standardized to ensure consistent results. When filters prepared from a 10 per cent solution of two different lots of nitrocellulose were used, however, a flow rate of 10 to 15 ml. per minute was observed for the large filters under 60 cm. mercury pressure. It has been found that membranes prepared from an 8 to 10 per cent solution of nitrocellulose were satisfactory for retaining most proteins.

The procedure for impregnating the thimbles is like that described by Kabat and Mayer (*supra*) who also give the directions for cleaning the alundum thimbles prior to use. The alundum extraction thimble is fitted with a rubber stopper and glass tubing as indicated in the diagram (figure 2), and coated with nitrocellulose. The coating is deposited by immersing the thimble in a solution of nitrocellulose in glacial acetic acid for approximately one minute while a vacuum is applied. A tight seal is formed between the stopper and

[1] From the Department of Agricultural Bacteriology, University of Wisconsin, Madison, Wisconsin.

[2] This study was made possible through a grant from the Committee on Medical Research and Therapy of the American Trudeau Society, medical section of the National Tuberculosis Association.

[3] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

the thimble if the assembly is immersed just past the junction. As the filter is withdrawn there is an excess of solution at the tip of the thimble, but this can be distributed evenly by inverting and rotating. As soon as the coating is uniform, the nitrocellulose is precipitated by submerging the assembly in a large volume of water. It is important that the assembly does not touch the sides of the container. After approximately an hour the

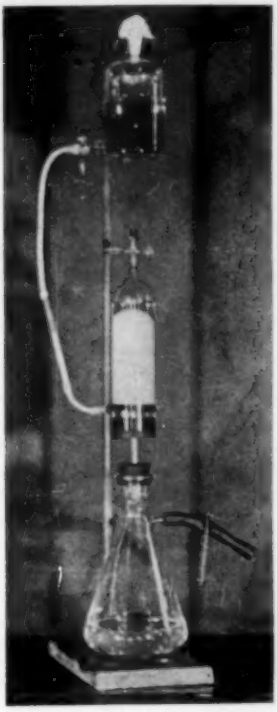

FIG. 1

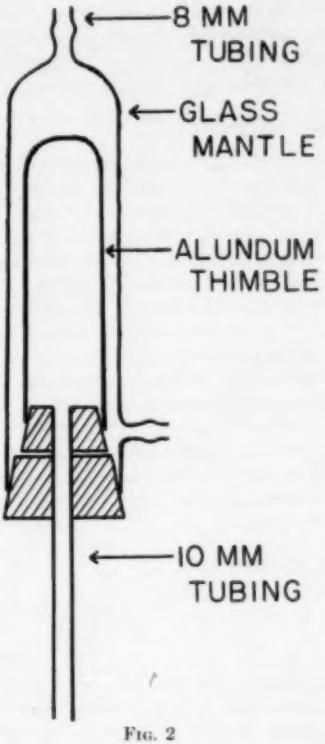

FIG. 2

FIG. 1. A photograph of an ultrafilter in operation
FIG. 2. A diagram of an ultrafilter assembly

membrane will have set sufficiently to permit placing it in the glass mantle. The second stopper should be adjusted so that the clearance between the filter and mantle is uniform. Once the filter is secured in the mantle, there is little chance for mechanical damage, and further washing is thereby simplified. The acid can be washed out of the filter quite rapidly by drawing water through it, and usually the pH of the effluent is nearly neutral after a liter has been passed through the filter.

Although a sterile assembly usually is not essential, it can be prepared with little difficulty. Since the nitrocellulose membrane is adversely affected by heating, it is necessary either to sterilize all of the component parts and to prepare the membrane aseptically, or

to sterilize the unit with a suitable disinfectant. The closed system makes it possible to maintain sterile conditions.

If the material being concentrated tends to deposit on the filter membrane as a gel, it usually can be resuspended easily without disassembling the filter unit by inverting or shaking the filter assembly.

Advantages of the use of the glass mantle are: It offers mechanical protection to the delicate nitrocellulose membrane; it provides a compact arrangement for ultrafiltration; the concentrated solutions can be withdrawn conveniently at any time during the filtration; the final volume of the concentrate can be very small; and the closed system makes it possible to maintain the sterility of both the filtrate and concentrate.

February 26, 1951

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POSSIBILITY OF SEXUAL CYCLE OF M. TUBERCULOSIS

To the Editors of the American Review of Tuberculosis:

All forms of life which have been adequately studied possess, in connection with their reproductive function, some mechanism for the combination of nuclear material from two individuals. There are many variations in this process, and in higher animals reproduction without a nuclear contribution from two individuals is impossible except under highly artificial conditions.

Genetic studies have demonstrated recombinations of characters in many bacteria (Lederberg, J.: J. Bact., 1950, *59*, 211) and even in some viruses (Hershey, A. D., and Rotman, R.: Genetics, 1949, *34*, 44). It would be strange indeed if tubercle bacilli were found to lack some sort of sex cycle. It should be possible to show conclusively by experiment whether or not recombinations of hereditary characters take place in mixed cultures.

If this does happen, such phenomena as the variations in development of drug resistance and the development of variants with different degrees of virulence could more easily be explained. Some weak spot might be found which would be vulnerable to a different therapeutic attack. BCG or other strains for immunization purposes might be kept more constant in their properties. Strains might even be developed for specific uses other than immunization. Strains with unique properties have already been isolated, but the mechanisms involved are not well understood.

Recently, new tools have become available. The electron microscope, the phase microscope, and even a combination microscope-television unit have been put into use. New micromanipulation instruments can overcome many earlier difficulties. Why cannot new light be focused on the private affairs of the tubercle bacillus?

February 21, 1951 — FLOYD M. FELDMANN

DIAGNOSTIC STANDARDS—1950 EDITION

To the Editors of The American Review of Tuberculosis:

In the 1950 edition of DIAGNOSTIC STANDARDS there is published the newly adopted clinical classification of pulmonary tuberculosis. Although I am pleased that the essential features of the Salkin-Cadden classification have been incorporated (J. Thoracic Surg., 1941, *11*, 126) there are several phases which call for some comment.

1. Although there is a good definition of a "negative bacteriology," including sputum, gastric juice, and bronchoscopic aspiration, there is no good definition of the term "roentgenologic evidence of cavity." Whereas a negative sputum implies that use was made of repeated cultures and guinea pigs, a roentgen survey still means (in most cases) a single or stereoscopic frontal view. A good X-ray examination should include a preliminary fluoroscopy plus any additional techniques that may be of value, such as obliques, lordotic and kyphotic views, lateral, over-penetration films, and planigrams. Any or all of these views may be necessary to determine the presence of cavitation and the nature of the lesion. If rarefied shadows are present suggestive of apical bronchiectasis, a bronchogram may be necessary. The roentgenographic study must be just as complete as the bacteriological, whether the sputum be positive or negative. Such X-ray studies may discover

otherwise hidden disease and explain a number of "arrested" cases with positive cultures or the occasional positive concentrate.

The close dependence of thorough bacteriological and roentgen studies is seen in daily practice in such examples: (*1*) Many rarefied shadows are considered cavities only if the sputum is positive and as a noncavernous lesion if the sputum is negative, unless shown otherwise by serial study; (*2*) the presence of a positive sputum without a definite cavity on the conventional films indicates the use of one or more supplementary views and planigrams in the search for cavitation; and (*3*) the existence of shadows suggestive of cavity in the presence of negative sputum usually indicates a more intensive and prolonged search for tubercle bacilli in sputum and gastric juice.

2. The term "arrested" in the new classification is not a happy one. Although its use suggests that the disease is inactivated, yet its new definition infers that active disease may be present and include such conditions as an improving lesion approaching true inactivity, an active lesion with a definite cavity but with sputum positive only on cultures or occasional smears, and an inactive parenchymal lesion but an active tuberculous bronchitis with only positive cultures. Many resections and thoracoplasties are being done on patients who have only positive cultures and cavity seen only on planigrams. For example, at the San Fernando VA Hospital, of the last 56 thoracoplasties done, 7 patients (12½ per cent) showed a positive sputum only on culture; of the last 17 cavernous lungs resected, 2 patients showed a positive sputum on culture only, and 2 patients showed cavity on planigrams only; the last four noncavernous resections showed highly active tuberculosis, yet the sputum was negative on smear and culture and X-rays showed "arrested" disease. Some other word meaning an approach to inactivity would be more suitable, viz., improved or quiescent.

3. The "exercise status" classification could have been simplified further. Instead of having five numbered groups with a vague conception of just how much activity the term "ambulatory" includes, it would have been simpler to use the following scheme, or some modification which would correspond more truly to the methods actually used in practice:

Bed rest group—varying from strict immobilization to bathroom privileges.

Semi-ambulatory—including a modified bed rest, meals in the common dining room, mass entertainment, et cetera, gradually reaching a total period out of bed to 4 hours.

Ambulatory—a further progression of activity such as planned walks, occupational therapy out of bed, shop work, and attendance at school classes.

Ordinary living conditions may include a return to former full activities or to a new life consistent with medical limitations.

In proposing our original classification of "active-improved-inactive" with a separate exercise classification, we were motivated by the fact that the 1940 classification was not only inadequate and confusing, but was also difficult to remember by the phthisiologist, as well as by the general medical profession. The current high level of bacteriological and roentgen diagnosis and therapy, coupled with the ever increasing public health aspects, demands a clinical classification that is both adequate yet simple enough to be understood by the specialist, nonspecialist, and patient. The new classification is a step in the right direction.

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March 2, 1951 DAVID SALKIN

BOOKS

MAURICE POROT: *La Psychologie des Tuberculeux. Pp. 211, Delachaux et Niestlé, Neuchatel, Switzerland, 1950.*

Anyone immersed in the 1951 struggle for devising and applying scales for the measurement of human behavior will need a little readjustment before he can appreciate the value of Doctor Porot's study which presents in excellent literary form the mature reflection of an acute clinical observer. Formerly Chief of the Medical Service of the Medical Faculty of Algiers and also trained in neuropsychiatry, Dr. Maurice Porot was himself a patient in a sanatorium for tuberculosis where he made long-continued observations on the psychology of his fellow inmates and assembled a bibliography of well nigh a thousand titles on the subject.

Fortunately for the American reviewer, Doctor Porot does not dwell at length upon the favorite European concept of the all-embracing and vague influence of the vegetative nervous system, and concedes the universal ignorance on the influence of the Koch bacillus on the psyche. The major portion of the book consists of the author's observations on the parallel course of tuberculosis and life situations in a number of patients; and the result is a sympathetic, sane, and penetrating account of the psychological problems occurring in the course and treatment of tuberculosis.

In describing life in the sanatorium as a vacation from life, Doctor Porot draws an analogy with the experiences of men suspended from their daily lives through deportation to labor camps and quotes freely from their writings, so that the reader who knows little of the human isolation resulting from chronic illness and prolonged separation cannot help but have his appreciation of the problem enhanced. Following through on the same theme, letters from patients document the statements that the problem of vocational re-education of a tuberculous individual is as nothing compared to the problem of his reintegration into family and community life.

Other penetrating observations accompanied by case histories concern the influence of grief prolonged and profound on the development and course of the disease, the development of egocentricity, hostility, pettiness, and susceptibility to the wiles of charlatans under sanatorium conditions.

Discussing sex, the author points out that the management of sexual impulses among young people condemned to a life of inactivity presents a real problem without one having to postulate any fantastic hypothesis. His observations on death and the desire to be deluded, which certain patients previously realistic about their condition manifest when the end is near, are both acute and poignant. Throughout, Doctor Porot points up the role of the physician in the management of tuberculous patients, and his observations cannot fail to be useful to the phthisiologist.

Mention is made of the necessity of considering surgery from the point of view of its psychological effect upon the patient but, although the book is published in Switzerland in 1950, there are no observations on the effect of the use of streptomycin on the psychology of the patients. Since most of the case reports cover a span of years of study, we hope that Doctor Porot is continuing his contacts and will publish reports on patients who have received streptomycin after they have been under his observation for several years.

BEATRICE BISHOP BERLE

ARTHUR J. VORWALD (editor): *Pneumoconiosis: Beryllium, Bauxite Fumes, Compensation. Sixth Saranac Symposium. Pp. 659, Paul B. Hoeber, Inc., New York, 1950, Price, $10.*

This memorial volume, dedicated to the late Dr. Leroy W. Gardner, who planned the symposium before his death, assembles a vast amount of information pertinent to beryllium and bauxite fumes as they relate to industrial health, together with a review of the latest developments on compensation for occupational diseases.

The first five sections (Parts I–V), each of which contains several papers followed by discussions, questions, and answers, are devoted to the beryllium problem and include the history and industrial aspects, the acute disease, the chronic or delayed disease, toxicity studies in connection with atomic energy production, and experimental aspects. Part VI considers the clinical aspects of Shaver's diseases developed among workers engaged in the processing of bauxite for the manufacture of corundum, and Part VII is concerned with compensation for occupational disease.

Throughout the volume are excellent graphs, tables, photographs, photomicrographs, and spectrograms. An extensive bibliography of 684 references up to January 1, 1950 and an excellent index of double column pages are found at the close of the volume.

The authors of the symposium are to be congratulated on this fine contribution to the present knowledge of two important, but still obscure, pneumoconioses and of the medico-legal problems involved.

W. J. MCCONNELL

JOHN J. HANLON: *Principles of Public Health Administration. Pp. 506, C. V. Mosby Company, St. Louis, Missouri, 1950, Price, $6.00.*

This text is a refreshing and much needed addition to the literature in the field of public health administration. Doctor Hanlon has introduced his subject in Part I of his book by a thoughtful account of the background and philosophy of public health administration. This is followed by Parts II and III which are devoted to administrative considerations in public health administration and the pattern of public health activities in the United States. The text especially intrigues the reviewer because of the fact that the author has presented so well the relationship which public health administration bears to public administration per se.

It is apparent that Doctor Hanlon believes that every health officer should have fundamental knowledge of the way in which government in general operates; and detailed, specific knowledge of the governmental unit of which his department is an integral part. It is believed that this concept is deserving of wider acceptance than is presently the case. This text should do much to promote this point of view.

In similar fashion the author portrays the relationship of public health administration to the social sciences such as sociology, economics, and so forth. In an especially fine way the text gives helpful information about the organizational structure of health departments, personnel matters, fiscal management, public relations, and the legal aspects of public health work. It is believed that the chapter which is devoted to public relations contains many practical suggestions which could be placed in operation with profit in many health departments. Every health officer will profit by the information which Doctor Hanlon so interestingly gives in regard to these facets of public health administration.

The final portion of the text is devoted to the consideration of the administrative as-

pects of the usual public health programs, such as control of communicable diseases, vital statistics, health education, environmental sanitation, maternal and child health, and so forth. These are succinctly and graphically portrayed from the administrative rather than the clinical viewpoint, which is proper in a text of this type.

The text is unusually well documented, covering not only the usual phases of public health administration, but also with reference to the fields of political science and sociology. It is believed that every alert health officer will profit by reading this book, and it is recommended to the thoughtful consideration of all students of public health administration.

RICHARD F. BOYD

RICHARD H. SWEET: *Thoracic Surgery. Pp. 345, W. B. Saunders Company, Philadelphia and London, 1950, Price, $10.*

Doctor Sweet has written an excellent book dealing with every aspect of the specialty of thoracic surgery stressing surgical anatomy, operative techniques, and postoperative care. It is a good guide for the graduate student of thoracic surgery as well as a reference for the thoracic surgeon himself. It is written with clarity and brevity but with due attention to the innumerable details which make for success in the performance of any surgical procedure.

The subject matter is arranged upon a regional anatomic basis beginning with the surgical anatomy of the thorax, general technical considerations, and thoracic incisions, and proceeding through operations on the chest wall, the pleural cavity, the lungs, the mediastinum, the heart and great vessels, the esophagus, the diaphragm, and abdominal operations performed through thoracic and diaphragmatic incisions. Appropriate applications of operative procedures are described as well as variations of technique to suit the particular pathologic process. Only those operations which the author has found useful in everyday practice are presented in detail. Those procedures which he considers to be in the early phases of development and not well established are omitted or mentioned only in passing. Prominent among these are mitral commissurotomy, and pulmonary or aortic valvulotomy.

The illustrations, done in the main by Dr. Jorge Rodriques Arroyo, are magnificent for their accuracy, simplicity, and realistic appearance. The drawings of surgical techniques were made from actual operations observed by Doctor Rodriques Arroyo while he was serving as a Fellow in Thoracic Surgery at the Massachusetts General Hospital.

The author makes the statement in his preface that "any properly qualified surgeon can acquire with relative ease a satisfactory proficiency in thoracic surgery by employing the technics" described in his book. There are those who feel that such a statement, although qualified, carries the dangers of over-simplification, and that something more is required to attain a satisfactory proficiency in thoracic surgery. Particular training and experience with thoracic diseases, including the natural history of these diseases, their endoscopic characteristics and diagnosis, and the normal and pathologic physiology involved, are mandatory requirements for the successful application of thoracic surgical techniques today. Doctor Sweet's book stresses operative techniques and does not profess to discuss thoracic diseases as such.

Doctor Sweet's book will be highly valued by those who appreciate his many contributions to the field of thoracic surgery.

DONALD L. PAULSON

G. MAURER: *The Chemotherapeutic Tamponade of Pulmonary Cavities (Die Chemotherapeutische Tamponade der Lungenkavernen). Pp. 114, with illustrations, Georg Thieme Verlag, Stuttgart, agents for U.S.A., Grune Stratton, Inc., New York, 1950.*

The author proposes what he believes to be a radically different treatment of cavitary pulmonary tuberculosis. The bacteriologic cleansing and continual observation of the pathologic and morphologic changes in the cavity take the place of the usual surgical attack. Where indicated, thoracoplasty may be added. Antiobiotics and chemotherapeutic agents are unsuccessful as treatment of cavitary pulmonary disease because of reduced permeability of the cavity wall and because the local vascular network is partly destroyed and partly thrombosed. The treatment by Maurer consists in essence of packing the cavity with gauze moistened with streptomycin or PAS. The most important points of the method are:

1. The "Speleostomie," i.e., the creation of a suitable opening into the cavity by stages, using laminaria bougies of gradually increasing size. These bougies are alternated with catheters until an eighteen-millimeter catheter can be inserted, an unbloody and aseptic method of opening the pulmonary cavity. Anesthesia of the intercostal nerves in the vicinity may be necessary because of pressure neuralgia.

2. Packing the cavity with gauze moistened with PAS. Periodic (six times daily) moistening of the external end of the pack is advised. The gauze acts as a wick to keep the cavity wall constantly in contact with the PAS. Conditions are created which simulate *in vitro* methods of destroying cultures of tubercle bacilli. Usually, seventeen days after the first tamponade the cavity secretions are free of bacilli and remain that way. The continuing improvement of the cavity in regard to its size and appearance is observed endoscopically with great clarity. It may be compared to the ophthalmoscopic observation of the healing process in tuberculosis of the eye.

3. Obliteration of the draining bronchi issuing from the cavity. This is accomplished by high-frequency coagulation. Usually all of the bronchial openings may be seen and reached without too great difficulty. The cavity must be completely clean and sterilized before the obliteration of the bronchi is carried out. The individual phases of the method are relatively easy, technically speaking. Serious complications are rare in the author's experience. The early results have been promising but final judgement must be reserved because insufficient time has elapsed since this method of treatment has been tried. Respiratory insufficiency and a free pleural space over the cavity are contraindications. Small cavitary processes cannot be treated by this method. To avoid the danger of severe bleeding only superficial cavities in the lower three-fourths of the lungs should be attacked. In the upper one-fourth, the anterior approach is desirable but the paravertebral approach may be utilized where necessary.

4. Using the described method, pulmonary cavities may be sterilized and frequently completely closed. Treatment lasts from six to ten months, and where necessary a small thoracoplasty may complete the treatment. A four-rib thoracoplasty is usually sufficient with the scapula tucked into a pocket inside the ribs to ensure proper collapse. A primary thoracoplasty usually destroys more pulmonary function, and the healing tendency in the cavity is not as good as with the author's method. The classical thoracoplasty will, in the author's opinion, be confined to the treatment of small cavitary processes; lobectomy will be limited to central or basal cavities; and pneumonectomy will have little place in the treatment of cavitary phthisis.

The reviewer believes that the author is using a very meticulous method of doing a cavernostomy, rather overburdened with detail. Indications are essentially as generally

practiced in the United States. The aim of producing and maintaining a good, large drainage canal is to be regarded with favor. Packing the cavity with gauze saturated with PAS or streptomycin would seem to be good practice and their simultaneous use systemically would probably be indicated. The obliteration of the draining bronchi has been the goal of much effort along those lines and the author's method has allegedly been quite successful in practice. More work on this aspect of cavernostomy would seem to be desirable.

HARRY BARELL

JUAN MAX BOETTNER: *Malformaciones Congenitas Broncopulmonares (Bronchopulmonic Congenital Defects). Pp. 107, with illustrations, Liberia y editorial "El Ateneo," Florida 340—Cordoba 2099, Buenos Aires, 1949.*

To define or to classify is an arduous task, and the clinician owes a debt of gratitude to the scholar who defines terms by which clinical experience may be described. This work of description and classification in the field of congenital pulmonary defects has been undertaken by Dr. Max Boettner, Director of the Department of Tuberculosis in Asuncion, Paraguay.

There have been numerous articles on the subject of the various pulmonary anomalies scattered through the medical literature during recent years, but the present author has gathered together under one head a great deal of information on this subject which makes his present work the nearest thing that we know to a definitive monograph on the subject.

In the present small volume, the anatomical findings in pulmonary and alveolar agenesis, pulmonary and bronchogenic cysts, and bullous emphysema are described and these conditions are discussed from the point of view of differential diagnosis, criteria for diagnosis and treatment.

The bibliography consists of ninety-six titles from the foreign and American literature which the author has reviewed critically. Most interesting are Doctor Boettner's own case presentations accompanied by well-reproduced roentgenograms and lipiodal studies which indicate careful study and observation. Since the North American reader is unfortunately limited in his knowledge of Spanish and since this volume may be considered as an authoritative reference on the subject of congenital pulmonary defects, it is unfortunate that Doctor Boettner has not summarized his definitions and observations on the several conditions described in one comparative chart.

BEATRICE BISHOP BERLE

BOOKS RECEIVED

PHYSICAL DIAGNOSIS. Fourth edition. By Ralph H. Major. Pp. 446 with 469 figures, W. B. Saunders Company, Philadelphia and London, 1951, Price, $6.50

PULMONARY VENTILATION AND ITS PHYSIOLOGICAL REGULATION. By John S. Gray. Pp. 82, Charles C Thomas, Springfield, Illinois, 1950, Price, $2.00

CURRENT THERAPY 1951—LATEST APPROVED METHODS OF TREATMENT FOR THE PRACTICING PHYSICIAN. Edited by Howard F. Conn. Pp. 699, W. B. Saunders Company, Philadelphia and London, 1951, Price, $10

TREPHINE TECHNIQUE OF BONE MARROW INFUSIONS AND TISSUE BIOPSIES. Fourth edition. By Henry Turkel. Pp. 60, Trephine Instruments Inc., 1301 Industrial Bank Bldg., Detroit, 1951, Price, $1.00

DIABETES GUIDE BOOK FOR THE PHYSICIAN. By The American Diabetes Association, Inc., 11 West 42 Street, New York, N. Y., 1951

OCCUPATIONAL FACTORS IN THE AETIOLOGY OF GASTRIC AND DUODENAL ULCERS, WITH AN ESTIMATE OF THEIR INCIDENCE IN THE GENERAL POPULATION. By Richard Doll and F. Avery Jones, with the assistance of M. M. Buckatzsch. Pp. 96, His Majesty's Stationery Office, London, 1951, Price, 2s. 6d

DIE TUBERKULOSE: IHRE ERKENNUNG UND BEHANDLUNG. Edited by Hellmuth Deist and Hermann Krauss. Pp. 754, with illustrations, Ferdinand Enke, Stuttgart, 1951, Price, geheftet DM 63; gebunden DM 68

HUMAN GENETICS. By Reginald Ruggles Gates. 2 volumes. Pp. 1,518, with illustrations, The Macmillan Company, New York, 1948, Price, $15

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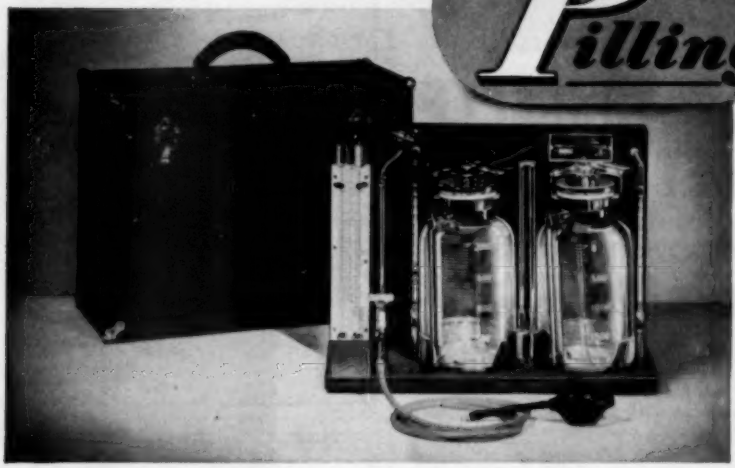

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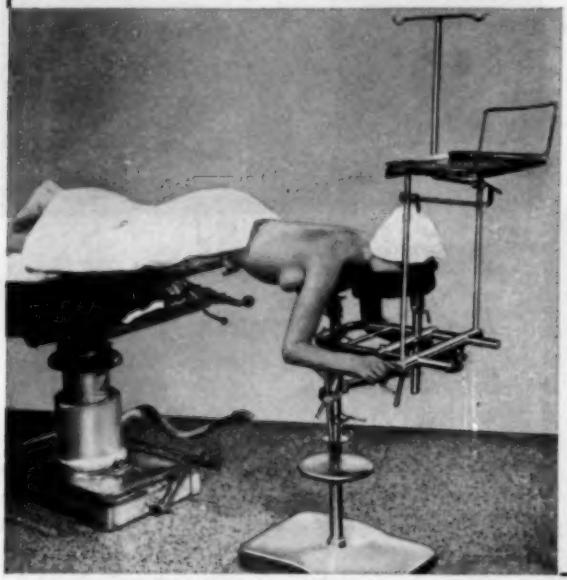

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SODIUM IN MEAT[3]

	Sodium Provided by 60 Gm. Serving	Sodium Provided by 100 Gm.
Beef, without bone	32 mg.	53 mg.
Lamb, without fat	66 mg.	110 mg.
Pork, without fat	35 mg.	58 mg.

Table I

SODIUM IN HOSPITAL DIETS[4]

Sodium-Poor Diets*				Very-Low-Sodium Diet†
40 Gm. Protein	70 Gm. Protein	100 Gm. Protein	130 Gm. Protein	70 Gm. Protein
400 mg. Na	500 mg. Na	800 mg. Na	1,000 mg. Na	200 mg. Na

Table II

*Foods prepared and served without salt.
†Weighed diet. May contain 4 oz. of unsalted meat.
(Normal diets contain approximately 4 Gm. of sodium daily.)

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1. Wheeler, E. O.; Bridges, W. C., and White, P. D.: Diet Low in Salt (Sodium) in Congestive Heart Failure, J.A.M.A. 135:16 (Jan. 4) 1947.
2. Wohl, M. G., and Schwartzman, N. G.: Diuretherapy (Cardiovascular Diseases), in Jolliffe, N.; Tisdall, F. F., and Cannon, P. R.: Clinical Nutrition, New York, Paul B. Hoeber, Inc., 1950, chap. 27.
3. Bills, C. E.; McDonald, T. C.; Niederpruem, W., and Schwartz, M. C.: Survey of the Sodium and Potassium Content of Foods and Waters by the Flame Photometer, Fed. Proc. 6:402 (Mar.) 1947.
4. Mayo Clinic Diet Manual, Philadelphia, W. B. Saunders Company, 1949, p. 115.

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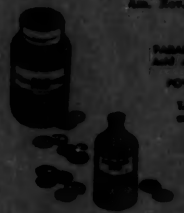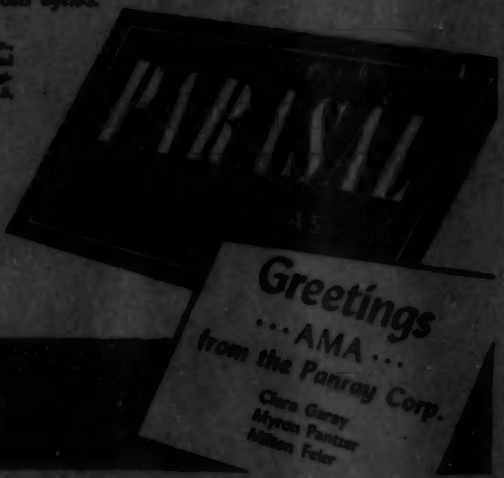
*TEMPEL, C. W., PRINDLE, J. H. Jr., MARDES, R. C., TOWERS, M. N., and DYE, W. E. Combined intermittent regimen employing streptomycin and PAS. Am. Rev. Tuberc., 1951, 63, 205